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Immunohistological Study of Elastin Protein Expression Among Iraqi Women Patients with Breast Carcinoma at Thi-Qar Province

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Science of Biology**

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سورة يوسف (الآية : 76)

DETICATION

**To whom suffer from breast cancer
with my wishes safety to them**

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SUMMARY

Immunohistochemical this study was to determine the correlation between the elastin protein expression with another steroidal markers such as estrogen receptors (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (Her2). All the experiment has been done in university of Thi-Qar \ College of science \ Biology department total sample (60) diagnosed as invasive ductal carcinoma specimens which have been to the all samples are obtained from patients with breast cancer during the period from November 2016 to June 2017, including (60) samples, all sample had obtained from the laboratory of Al-Hussein teaching Hospital in Thi-Qar providence.. the mean of the age of all patients was 50 years, all the samples have as the grade >2 , stage >3 and scores >3 .

The skin (section of breast)) was the positive control, it has been appeared a highly elastin protein expression.

Significant positive correlation have been found between the elastin protein expression and the age, the value of correlation was (0.45) at significant $p \leq 0.01$, this study has been proved the significant positive correlation $p \leq 0.01$, between the elastin protein expression in the all breast invasive ductal carcinoma with ER+, PR+ expression, it was 0.235 and 0.254 respectively. Also this study has shown a significant positive correlation at $p \leq 0.01$ between the elastin expression and the positive ER and PR expression, it was 0.312 and 0.355 consecutively.

Another outcome in this study was (-0.038, -0.299) Consecutively negative correlation between the Her2 expression prognostic marker with ER and PR. finally, a significant positive strong correlation between the expression of ER and PR prognostic parameters at the level $p < 0.01$, the value of this correlation was 0.541.

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Abbreviation	Symbol
Americana Cancer Society	ACS
Age Standardized Incidence Rates	ASIR
Breast Cancer	BC
Carcinoma Associated Fibroblasts	CAFs
Ductal Carcinoma In Situ	DCIS
Disteren Plasticizer Xylene	DPX
Extracellular Matrix	ECM
Estrogen Receptor	ER
Figure	Fig
Human Epidermal Growth Factor Receptor 2	Her2
The Heat-Induced Epitope Retrieval	HIER
Hormone Replacement Therapy	HRT
International Agency For Cancer Research On Cancer	IACR
Inflammatory Breast Cancer	IBC
Invasive Ductal Carcinoma	IDC
Invasive Lobular Carcinoma	ILC
Iraqi National Cancer Research Center	INCRC
Lobular Carcinoma In Situ	LCIS
National Cancer Institute	NCI
Oral Contraceptives	OCS
Photolytic-Induced Epitope Retrieval	PIER
Progesterone Receptor	PR
World Health Organization	WHO

1.1 Introduction

The Cancer is an abnormal growth of cells which tend to proliferate in an uncontrolled way, sometimes to metastasize (spread). It is not one specific type of diseases. It is in fact over one hundred different types of it. Cancer can be occurs in any tissue of the body and have many forms in each area in the body (National Cancer Institute, 2014).

The breast cancer is an important disease because of wide separation over all worlds and it has highly death rate. It has effect on women under forty five years old (Samphao *et al.*, 2009).

Breast cancer is the most common malignancy and the due to cause of death in women, with more than 1, 00,000 cases occurring worldwide annually (Axelrod *et al.*, 2008). Over the world, breast cancer consist of 10.4% of all cancer incidence among women, making it the most common type of non-skin cancer in women and the fifth most common cause of cancer death (World Cancer Report, 2003). In 2004, about 519,000 deaths worldwide had been caused by breast cancer (7% of cancer deaths; almost 1% of all deaths) (Fact Sheet No. 297: 5).

Elastic fibers (or yellow fibers) are bundles of [proteins](#) ([elastin](#)) found in [extracellular](#) [matrix](#) of [connective](#) [tissue](#) and produced by [fibroblasts](#) and [smooth muscle](#) cells in [arteries](#). These fibers can stretch up to 1.5 times their length, and snap back to their original length when relaxed. Elastic fibers include [elastin](#), [elaunin](#) and [oxytalan](#) (Liu *et al.*, 2004)

Elastic tissue is classified as connective tissue proper, it is formed from the elastic micro fibril (consisting of numerous proteins such as microfibrill a associated [glycoprotein's](#), [fibrillin](#), [fibullin](#), and the [elastin receptor](#)) and amorphous [elastin](#) (Strum *et al.*, 2007).

In skin elastic fibers constitute a relatively small proportion, 2-4 %, of skin dry weight. Despite their comparatively low quantity, they are responsible for the resilient and elastic properties of skin. The elastin protein was the main constituent of the elastic fibers present in extracellular (Kielty, 2002) .

Elastin is a highly [elastic protein](#) in [connective tissue](#) and allows many tissues in the body to resume their shape after stretching or contracting. elastin helps skin to return to its original position when it is poked or pinched. elastin is also an important load-bearing tissue in the bodies of vertebrates and used in places where mechanical energy is required to be stored (Almine *et al.*, 2010).

The first immunohistochemistry markers evaluated in breast cancer are Estrogen receptor (ER) and progesterone receptor (PR). the presence of these markers in tumor tissue correlates with good response to the chemo and hormone therapy (Barnes *et al.*, 2001). Estrogen and progesterone receptors and HER-2 have important influence the management of the malignancy (Rampaul *et al.*, 2001; Mori *et al.*, 2002).

In addition, there are another factors such as age, tumor size, lymph node involvement, histologic type, and tumor grade at the time of initial diagnosis has been established as a clinically useful, standard of care parameter in determining treatment options and subsequent patient response (Gown, 2008) .

The aims of the study to :

- 1- Clarified the elastin protein expression in patients with breast cancer.
- 2- Identified the relationship between elastin protein expression and steroidal hormone receptors (ER, PR and Her2) in breast cancer patients.

3- Determined the correlation between elastin protein expression ER, PR and Her2 with the age factor.

2.1 Breast Cancer

Breast cancer is a complex and multi factorial disease resulting in abnormal cell growth that leads to malignant tumor formation, breast cancer in women is a worldwide, it is the fifth most common cause of cancer mortality (Bray *et al.*, 2012). Iraqi National Cancer Research Center (INCRC) considered breast cancer as first type of Iraqi female cancer, there are approximately about (1:3) of the account registered is female cancers in Iraq (INCRC, 2013).

There are five major cancer causing overall mortalities per year: by the- Lung: 1.3 million, Stomach: 1 million, Liver: 662, 000, Colon: 655,000 and Breast: 502,000 compare with men the lung cancer is the number one then stomach, liver, Colorectal and esophagus while the women are the- breast cancer is the number one then, lung, stomach, colorectal, and cervical, According to the International Agency for Cancer Research on cancer (IACR), the Age Standardized Incidence Rates (ASIR) in Iraq was (31.1) years, similar as compared to the Iraq surrounding, countries, Kuwait (47.7) years, Saudi Arabia (22.4) years, Jordan (47.0) years, Syria (23.0) years, Iran (18.4) years, Turkey (28.3) years (Globocan, 2008).

The sub-types of breast cancer ; is about (16) some cancers Proliferate from pre-invasive lesions (World Cancer Report, 2014), According to the- Iraqi Cancer Registry in 2008, BC is the commonest type of female malignancy, it is accounting approximately one-third of the registered female cancers (Iraqi Cancer Board, 2004).

2.2 Types of breast carcinoma

The breast cancer was classified into many subtypes according to the breast part (Fayed, 2009).

1- Ductal Carcinoma in situ: starts in the cells which line the breast's ducts, beneath the nipple and areola. The ducts supply milk to the nipple. Between 85% and 90% of all BC are ductal. if the cancer is DCIS (ductal carcinoma in situ), it is well contained, not invasive, and can be very successfully treated.

2- Lobular Carcinoma in situ : begins in the lobes, or glands which produce milk in the breast. The lobes are located deeper inside the breast, under the ducts. only 8% of breast cancer are regarded as lobular. if the cancer is LCIS (lobular carcinoma in situ) that means the cancer is limited within the lobe and has not spread.

3- Invasive (Infiltrating) ductal carcinoma (IDC) in situ : Invasive or infiltrating, it is the most common type of, about 80% of a breast cancers are invasive ductal carcinoma. Breast cancer has the potential to spread out of the original tumor site and invade other parts of breast and body. there are several types and subtypes of invasive breast cancer, According to the American cancer society more than 180.000 women are diagnosed with invasive ducal carcinoma.

4 - Inflammatory Breast Carcinoma (IBC) in situ : a rare and most aggressive of breast cancers, taking the form of sheets or nests, instead of lumps. IBC tends to grow and spread quickly with symptom worsening within days or even hours. this kind of cancer usually start with Redding and swelling of the breast instead of a distinct lump. unlike ducal and

lobular cancers, it is treated first with chemotherapy and then with surgery.

- **Paget's disease** of the nipple/areola least common is a cancer of the nipple, often looks like a skin rash, or rough skin. It resembles eczema, 13 and can be itchy. The itching and scabs (if scratched) are signs that cancer may be under the surface of the skin.

2.3 Classifications

In general the tumors are classified according to the most differentiated cells with the exception of carcinoma where a few tumors cells show neuroendocrine differentiation. the classification of breast tumors which includes benign tumors and malignant tumors, according to following pathological types (Viale, 2012):

A- Non-invasive

- Ductal carcinoma in situ (DCIS).
- Lobular carcinoma in situ (LCIS).

B-Invasive (infiltrating)

1. Invasive ductal carcinoma (IDC).

- Adenoid cystic carcinoma.
- Low-grade adenosquamous carcinoma
- Medullary carcinoma
- Mucinous carcinoma
- Papillary carcinoma
- Tubular carcinoma
- Metplastic carcinoma
- Micro papillary carcinoma

2-Invasive lobular carcinoma

- Classic carcinoma
- Pleomorphic carcinoma
- Tubule-alveolar carcinoma

3. Mixed ductal and lobular carcinoma

4. Undetermined or unclassified breast carcinoma

Almost types of breast cancer are invasive, or infiltrating. It is started in the-lobules or ducts of the breast then it break the duct glandular walls then invade the surrounding tissue of the breast (Ferlay *et al.*, 2010).

2.4 Breast anatomy

In women, the breasts overlay the [pectoralis major muscles](#) and usually extend from the level of the second rib to the level of the sixth rib in the-front of the [human rib cage](#) thus, the breasts cover much of the chest area and the chest walls. At the front of the chest, the breast tissue can extend from the [clavicle](#) (collarbone) to the middle of the [sternum](#) (breastbone). at the sides of the chest, the breast tissue can extend into the [axilla](#) (armpit), and can reach as far to the back as [dorsi muscle](#), extending from the lower back to the [humerus](#) bone (the longest bone of the upper arm). as a [mammary gland](#), the breast is composed of differing layers of [tissue](#), predominantly two types: [adipose tissue](#) and [glandular tissue](#), which affects the lactation functions of the breasts (Drake *et al.*, 2005). [morphologically](#) the breast is tear-shaped (Love and Susan, 2015) the- superficial tissue layer ([superficial fascia](#)) is separated from the skin by 0.5–2.5 cm of subcutaneous fat (adipose tissue). the [suspensory Cooper's ligaments](#) are fibrous-tissue prolongations that radiate from the superficial fascia to the skin envelope (see fig 2-1). the female adult breast contains 14–18 irregular lactiferous lobes that converge at the nipple. if 2.0–4.5 mm milk ducts are immediately surrounded with dense

connective tissue that support the glands. milk exits the breast through the nipple, which is surrounded by a pigmented area of skin called the areola. the size of the areola can vary widely among women. the areola contains modified [sweat glands](#) known as [Montgomery's glands](#). these glands secrete oily fluid that lubricate and protect the nipple during breastfeeding (Stöppler *et al.*, 2015) Volatile compounds in these secretions may also serve as an olfactory stimulus for the newborn's appetite (Doucet *et al.*, 2009). the- dimensions and weight of the breast vary widely among women. a small-to-medium-sized breast weighs 500 grams (1.1 pounds) or less, and a large breast can weigh approximately 750 to 1,000 grams (1.7 to 2.2 pounds) or more. the tissue composition ratios of the breast also vary among women. Some women's breasts have varying proportions of glandular tissue than of adipose or [connective](#) tissues. the fat-to-connective-tissue ratio determines the density or firmness of the breast. During a woman's life, her breasts change size, shape, and weight due to hormonal changes during [puberty](#), the [menstrual cycle](#), pregnancy, breastfeeding, and menopause(Pamplona *et al.*, 2004).

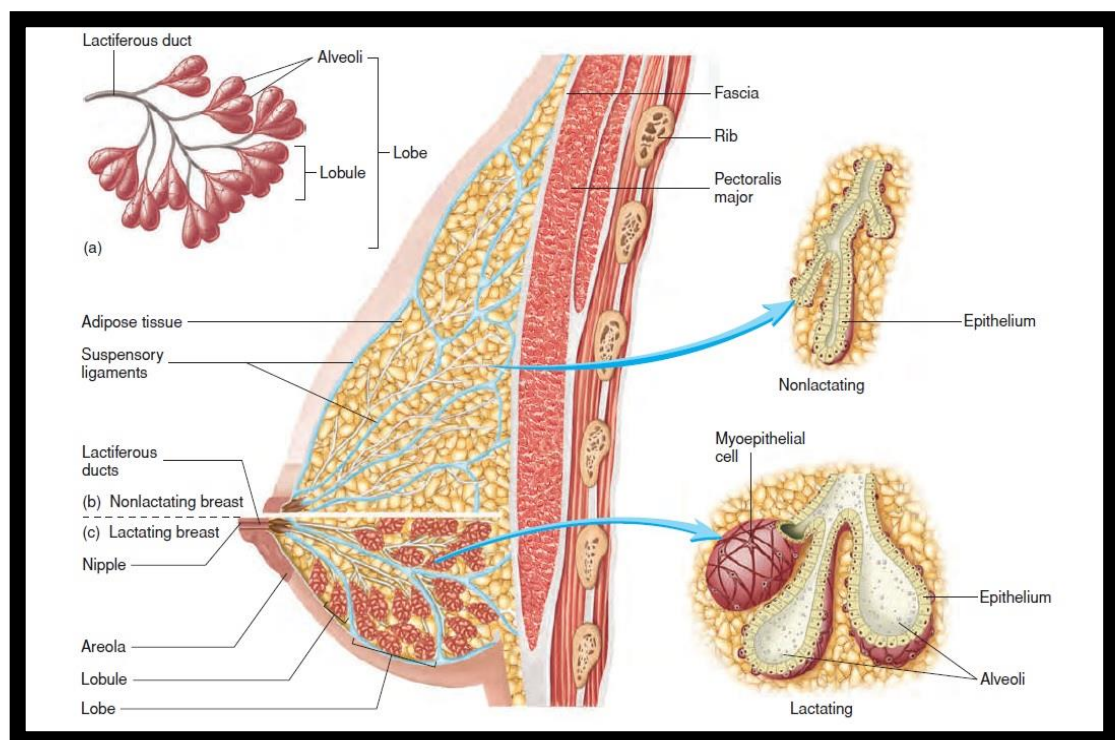


Fig (2-1): Breast Anatomy, (a) Each lactiferous duct of the mammary gland branches, at the end of each branch is one or more alveoli. **(b)** the nonlactating breast has a duct system that is not extensively developed . The branches of the lactiferous duct end as small, tube like structure. **(c)** the lactating breast has a well-developed duct system with many branches, the branches of the lactiferous duct end with well-developed alveoli, adipose tissue is abundant in both the nonlactating and the lactating breast.

(VanPutte *et al.*, 2017) .

2.5 Epidemiology

Breast cancer is the most frequent cancer in women worldwide, representing 1.38 million new cancer cases diagnosed in 2008, constituting 23% of all cancer cases in women. It is now the most common cancer in developing countries, the range of mortality rates (by about 6-19 per 100,000), in 2013, an estimated 232,340 women were expected to be diagnosed in the US with breast cancer with 39,620 women were expected to die (ACS, 2013).

Incidence rates vary from 19.3 per 100,000 women in Eastern Africa to 89.7 per 100,000 women in Western Europe, and are high (greater than 80 per 100,000) in developed regions of the world (except Japan) and low (less than 40 per 100,000) in most of the developing regions (Ferlay *et al.*, 2010).

2.6 Effect of risk factors on breast cancer

1-sex: Simply being a woman is the main risk factor for developing breast cancer Women's breast [duct cells](#) are more developed than men's,

and women's breast cells are constantly exposed to the female [hormone estrogen](#), which is essential to women's normal growth, development and reproduction. exposure to estrogen is a factor in the development of breast cancer , men can develop breast cancer , but this disease is about 100times more common among women than men (ACS, 2012) .

2. Age: The strongest risk factor for breast cancer (after gender) is age: the older women, are higher risk, the incidence of breast cancer increases with age, doubling about every 10 years until the menopause, when the rate of increase slows dramatically (CGHFBC, 2002).

3. Reproductive history: Women in developed countries are at increased risk of breast cancer compared with women from less developed countries, much of this variation can be explained by the fact that women in developed countries have fewer children and a limited duration of breastfeeding (Clavel-Chapelon *et al.*, 2002)

2.6.1 Reproductive Factors Influence Breast Cancer Risk

Studies have shown that a woman risk of developing breast cancer is related to her exposure to hormones are produce by her ovaries, reproductive factors that increase the duration and / or levels of exposure to ovarian hormones, which stimulate cell growth, have associated with an increase in breast cancer risk, conversely, pregnancy and breast feeding associated with a decrease in breast cancer risk(Kelsey et al., 1993).

2.6.1.1Age at menarche: Early age at menarche has been consistently associated with an increased risk of breast cancer. Average age of menarche in developed countries fell from around 16-17 years in the mid

19th century to 12-13 today, Relative risk for premenopausal breast cancer is reduced by an estimated 7% for each year that menarche is delayed after age 12 years, and by 3% for postmenopausal breast cancer , Low risk countries such as China have a later average age at menarche (16-17 years) (Holmberg *et al.*, 2005).

2.6.1.2 Age at first birth: The younger the woman is when she begins childbearing, the lower her risk of breast cancer . every each year of delay the relative of breast cancer happen by 3%(CGHFBC, 2002).

2.6.1.3 Breast feeding: women who depend on breast feeding reduced the exposure to risk of breast cancer compared to women don't depend breast feeding. The women with long are greater the protection, risk is reduced by 4.3% for each year a woman breastfeeds (Walker, 2008).

2.6.1.4 Age at menopause: Late the women have late menopause increases the risk of breast cancer . for each year menopause is delayed, there is an approximate 3% increase in breast cancer risk, Postmenopausal women have a lower risk of (BC) compared to premenopausal women of the same age. this is true for both natural menopause or menopause induced through surgery (Oran *et al.*, 2004).

2.6.1.5 Exogenous Hormones :The use of oral contraceptives (OCs) slightly increases the risk of (BC) in current and recent users, but there is no significant excess risk ten or more years after stopping use the risk of oral contraceptive (Althuis *et al.*, 2003).

2.6.1.6 Physical Activity: the physical activity is a preventive effect on breast cancer. this may be an indirect effect with exercise lowering body mass index. or a direct effect on hormonal and growth factor levels (WHO, 2002).

2.6.1.7 Alcohol Intake: A significant association between alcohol intake and (BC) has been found, with an increase of risk 7% for each additional 10 grams of alcohol consumed on a daily basis .Around 4% of breast cancers in women in developed countries may be attributable to alcohol. although alcohol and tobacco smoking are closely related social habits, there is no direct association between tobacco and (BC) (Baglietto *et al.*,2005).

2.6.1.8 Diet: A diet high in fat has been positively associated with breast cancer in international correlation studies, overall, the evidence suggests fat intake, particularly animal fat, may cause a small increased risk of breast cancer but probably does not play as large a role as was once thought (Hunter and Willett, 1993).

2.6.1.9 Breast Density: Density relates to the relative amounts of fat, connective tissue and epithelial tissue in the breast, breast with a higher proportion of fatty tissue are less dense. cancer is less easily detected in denser breasts. Women with denser breasts have 2-6 times the risk of (BC) compared to women with less dense breasts (Kerlikowske *et al.*, 2005).

2.6.1.10 Benign breast disease: Benign breast disease is a generic term describing all non-malignant breast conditions, as such it encompasses diseases associated with an increased risk of (BC) and others that do not have a raised risk (Boyd *et al.*, 2002).

2.6.1.11 Family History of Breast Cancer: A woman with one affected first degree relative (mother or sister) has approximately double the risk of (BC) a woman with no family history of the disease if two (or more) relatives are affected, her risk increases further. However, over 85% of women who have a close relative with (BC) will never develop the-

disease, and more than 85% of women with 19 (BC) have no family history of it (Silvera *et al.*, 2005). In developed countries it is estimated hereditary factors contribute around a quarter of inter individual differences in susceptibility to (BC), while environmental and lifestyle factors contribute the remaining three-quarters, a small proportion of women has a particularly strong family history of (BC) and are at very high risk, Women who carry mutations of BRCA1 (breast cancer gene 1) or BRCA2 (breast cancer gene 2) are at higher risk of developing both breast and ovarian cancer than women who do not have these genetic mutations (Malone et al., 1998).

2.7 The skin is the largest organ of the body, accounting for about 15% of the total adult body weight. It performs many vital functions, including protection against external physical, chemical, and biologic assailants, as well as prevention of excess water loss from the body and a role in thermoregulation. The skin is continuous, with the mucous membranes lining the body's surface, The integumentary system is formed by the skin and its derivative structures (see Figure 2-2). The skin is composed of three layers: the epidermis, the dermis, and subcutaneous tissue (Kanitakis, 2002)

The outermost level, the epidermis, consists of a specific constellation of cells known as keratinocytes, which function to synthesize keratin, a long, threadlike protein with a protective role. The middle layer, the dermis, is fundamentally made up of the fibrillar structural protein known as collagen. The dermis lies on the subcutaneous tissue, or panniculus, which contains small lobes of fat cells known as lipocytes. The thickness of these layers varies considerably, depending on the geographic location on the anatomy of the body. The eyelid, for example, has the thinnest layer of the epidermis, measuring less than 0.1 mm,

whereas the palms and soles of the feet have the thickest epidermal layer, measuring approximately 1.5 mm. The dermis is thickest on the back, where it is 30–40 times as thick as the overlying epidermis (James et al., 2006).

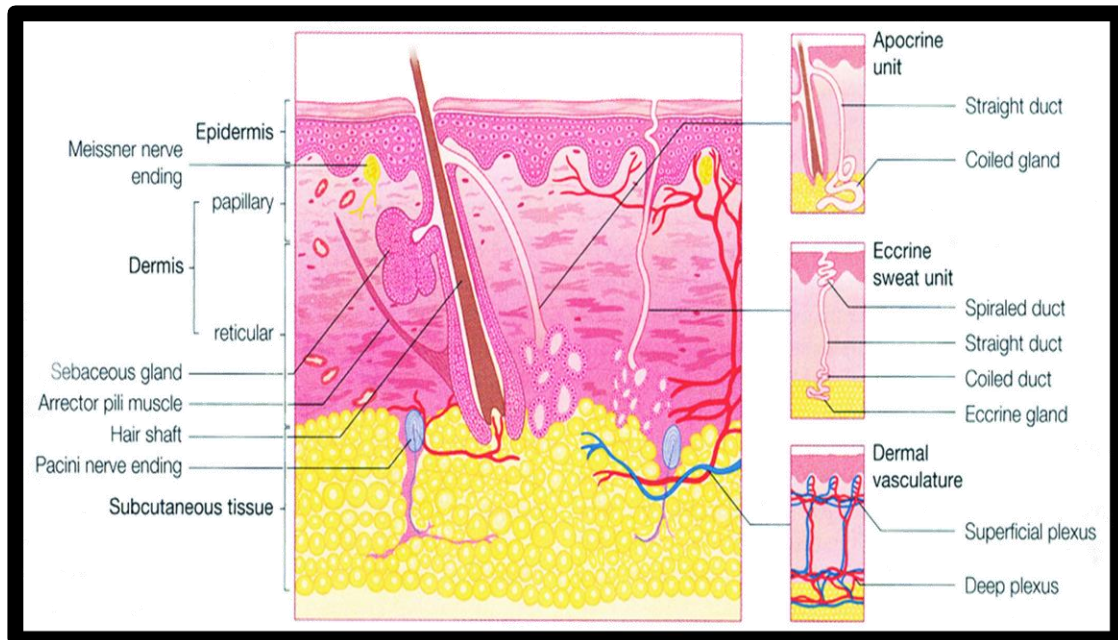


Figure (2-2): Cross-Section of Skin (James et al., 2006)

2.8 The extracellular matrix (ECM) constituents in malignant breast cancer

The ECM of tissues change under a lot of condition like during the pre adult development, estrous cycle pregnancy, lactation and involution

extracellular matrix .Also the extracellular matrix has remodeling during cancer occur (lochter and Bissel, 1995), summarized fellow :

2.8.1 ECM and tumor development

There are changes in composition and organization of ECM during breast carcinoma when compared to the mammary gland under homeostasis, the patients with (BC) exhibit different clinical outcome according to the ECM composition (Bergamaschi *et al.*, 2008).

There are many of ECM proteins when it had induced ,they will play a major functional role in progression and metastasis of (BC) , these proteins are included, fibrillar, specific laminins ,fibronectin, collagens and matricellular proteins as well as proteoglycans (Insua-Rodríguez and Oskarsson, 2016).Also there are major changes in composition of (ECM) in (BC) with altered expression such as positive expression in Elastin, Fibronectin, vitronectin and anhor positive expression markers, in the same time there are some negative markers expression in breast tumors and carcinoma cell such as type IVcollagen, Dermatan sulfate and heparin sulfite (Ioachim *et al.*, 2002) .

Some studies have been shown that the high fibronectin and elastin expression have been good positive correlated with good prognosis and decrease of metastasis in (BC) tissues in patient with (BC) (Kojima *et al.*, 2010) .

2.8.2 Tumor cell behavior

The tumor cells have some properties of unicellular organism (Shubic, 1994). The first of them is breakup their tight junctions with next cells, have motile function , and have path through surrounding obstacles like basement membrane and surrounding extracellular matrix, this mechanism which is associated with changes in the expression of cytoskeleton proteins, cell adhesion molecules, matrix degrading proteases and integrins valves a crosstalk between cancer cell and tumor stroma (Haslam, 1990 ; Sakakura, 1991) .

2.9 The elastin

Elastin, the major protein component of the elastic fiber, is an extracellular matrix protein of vertebrate that require elasticity such as the- arteries, lungs, skin and bladder. elastin plays an important role in providing these tissues, and others, the ability to stretch while maintaining healthy cells (Almine *et al.*, 2010). elastin is essential for proper arteriogenesis and airway development (Wendel *et al.*, 2000). also it has a significant role to ageing of connective tissue, the elastin gene has been isolated and localized on chromosome 7 in humans. studies was showed that the elastin gene expression initiates during early embryonic stages and remains relatively constant for several decades (Yamauchi *et al.*, 2010) .

The elastin synthesis typically occurs in fibroblast, vascular smooth muscles cells, and chondrocyte (Mithieux *et al.*, 2004). the level of elastin expression has been observed after injury and after pathogenic condition also the elastin expression is affected by elastase degradation of extracellular matrix have been show there are the both of tropoelastin and elastin degradation products are able to in fluence cell fucnction and

promote cellular responses such as proliferation and cell adhesion (Rodgers and Weiss, 2005) .

Elastin is a high insoluble polymer composed of covalently cross-linked molecules of its precursor, tropoelastin, a soluble, nonglycosylated and highly hydrophobic protein (Rodgers and Weiss, 2005) .

Elastin is present in good quantities in some organs like skin, large arteries and lung; it can also be present in special connective tissue (skeletal connective tissue) such as cartilage. Fibroblasts, smooth muscle cells and chondrocytes produce it as a 72 kDa precursor tropoelastin, which is secreted into the extracellular matrix (Chen *et al.*, 2014).

The elastin receptor has been reported by Lapis and Timmer (2002) as elastin-binding proteins (EBP). S-gal/EBP, the stimulation of this receptor can effect on the proliferation, chemotaxis, and adhesion of cells (Bax *et al.*, 2009). Lapis and Timmer (2002) have mentioned that the soluble elastin as well as elastin peptides is a strong inhibitor of cell metastasis in experimental tumor models.

Elastin as have shown in previous review, it is the main protein in large elastic blood vessels, where it is likely to inhibit the proliferation of vascular smooth muscle cells and so preventing vessel occlusion (Ito, 1998). The studies of (Rodgers and Weiss 2004) and (Broekelmann *et al.*, 2005) have shown that the tropoelastin of human and bovine can bind to cell surface receptors in many cell types directly. Also it binds directly to cells through ECM proteins (Yanagisawa *et al.*, 2002; Nakamura *et al.*, 2002).

The studies of (Karnik *et al.*, 2003) have shown that the binding of elastin to the elastin-binding proteins (EBP) made the cell

morphological changes , chemotaxis (Senior *et al.*,1982), decreased cell proliferation (Jung *et al.*,1998), and angiogenesis (Robinet *et al.*, 2005).

The result of these studies could be submit a good role of elastin peptides, it can be used to design targeted therapies by exploit the nature of this protein (Lapis and timer 2002).

During aging, the elasticity of connective tissue is reduced. elastin and elastase in human breast cancers increas linearly with age (Hornebeck *et al.*,1986).

Elastin in breast cancer can observed as both isolated singal fibers or large aggregates fibres in the stroma , around ducts or small blood vessels. The origin of this material comes from stromal cells like fibroblasts and myofibroblasts (Nakanishi *et al.*, 1983), also it synthesis by the-carcinoma cells (Kadar *et al.*, 2002).

2.9.1 Elastosis

Medical definition of elastosis is a condition marked by thickening and degeneration of elastic fibers and loss of elasticity in connective tissue.

Some researchers such as(Shivas and Douglas1972) had studied the-elastosis at first time in (BC) about decades ago, showing that present of large amounts of the elastotic material in tumors makes it have good a prognosis (Azzopardi and Laurini, 1974). Next studies have been focus on the correlation between the expression of estrogen receptor and present of elastosis (Masters *et al.*,1976; Glaubitz *et al.*, 1984).

The study of Tamura and Enjoji (1988) on the elastosis in non-invasive patients with invasive ductal carcinoma, they found a significant

correlation between the increased amount of elastic fibers in the non-neoplastic tissue, of the neoplasm and an increase in parity.

2.10 Steroid receptors and human epidermal growth factor receptor (HER)-2/neu

Estrogen receptors are a group of proteins found inside cells. they are receptors that are activated by the hormone estrogen (17β -estradiol).

The another Steroid receptors is the progesterone receptor (PR), is a protein found inside cells. it is activated by the steroid hormone progesterone. it also also known as NR3C3 or nuclear receptor .

Estrogen and progesterone receptors (ER, PR) and more recently, the human epidermal growth factor receptor 2 (HER2) has been found to be of prognostic and therapeutic importance in (BC), it is increasing importance influenced the management of the malignancy (Rampaul *et al.*, 2001; Mori *et al.*, 2002; Gown *et al.*, 2008).

The over expressions of ER, PR and HER2 are associated with clinical results and they are the prognostic factors and important predictive (Zhu *et al.*, 2014).

3. Materials and Methods

3.1 Material

The chemicals and instruments which have been used in this study are listed in table below.

Table (3-1): instruments and materials which are used in histological preparation.

Instruments And Material	Origin / Company
Hot Plate	Japan
Chloroform	Germany.
Cool Plate	Japan
Cover Slide 24*50 Mm	Japan
Disposable knives	Japan
Distilled water	Iraq
DPX	China
Forceps	Japan
Formalin	U.K
Heamatoxylin and Eosin	India
Isopropanol	U.K
Light microscope	China
Microtome	Jaban

Paraffin wax	Germany
Positive Charge Slides	(Afco).China
Slide	Japan
Staining Jar and Slide Rack	Japan
Xylene	Germany

3.2 Collection of specimens

The specimens of this study has been obtained from 60 patients diagnosed with (BC) were assayed for elastin protein expression estrogen receptors (ER) expression, progesterone receptors (PR) expression and human epidermal growth factor receptor-2 (HER2), the scoring of this parameters have been correlated with them age, add to the all samples are obtained from patients with (BC) during the period from November 2016 to January 2017, including (60) samples, all sample had obtained from the laboratory of Al-Hussein teaching Hospital in Thi-qar providence.

Section of human skin were used as appositive control, negative control were obtained by omitting the primary antibody .

3.3 Histological preparations

In the histological technique the preparation of tissue has been used according to (Luna, 1968) .

3.3.1 Histological Technique

3.3.1.1 Fixation

all samples from patients with (BC) instantly fixed in 10% formalin. The fixation time was about 22-24 hours at room temperature. Long fixation time (over 24 hours) usually yielded less accurate Immunohistochemical results.

3.3.1.2 Dehydration

A grade of isopropanol (30, 50, 70, 80, 90 and 100) for 2hours for each step was used to dehydrating tissues in an ascending .

3.3.1.3 Clearing

After dehydration, tissues cleared with chloroform for 12-24 hours, The chloroform is high commend clearing agent .

3.3.1.4 Impregnation

This process was done by using paraffin wax at 60 C for 12 hours. In

the first two hours the wax was twice renewed to remove the chloroform.

3.3.1.5 Embedding

The step have been done by using molten paraffin wax, by using warm forceps to put the specimens in the cleaned mold full with molten paraffin.

3.3.1 6 Sectioning

By using rotary microtome of first the blocks had trimmed for removing excessive wax and then sectioned 4 and 5 μm thickness section the ribbons were floated at warm water (35-40 C°) bath and mounted on clean slides with edge covered with a thin layer of Mayer's albumin after their extension in water bath and left to dry at room temperature. another group of slides made by using positive charge slides .

3.3.1.7 Haematoxlin and Eosin Staining

To have good staining paraffin must to be removed by xylene for 10 minutes, with using hot plate at 35C° in cold weather . sections were passed by descending concentration of ethyl alcohol absolute (90,80,70,50,30) for 2 minutes for each concentration ,stained with haematoxylin stain for 10 minutes and extensively washed with tap water .after that they were passed by ascending grade of ethanol (30,50,70,80,90,100) for 2 minutes each, stained with alcoholic eosin Stain for 2 minutes and then passed by passed by absolute ethanol for 5 min. finally sections were cleared by xylene (5-10) min. mounted by DPX ,covered with cover slides and removed to dry at room temperature to be ready for microscopic examination by using light microscope .

3.4 Detection of (ER, PR, Her2 and Elastin Protein) By Immunohistochemical Technique

3.4.1 Preparation of Tissue sections

Paraffin embedded section of (BC) tissues were cut into 4 μm thicknesses using a microtome . The section were applied on fisher-brand positively charged slides and left overnight to dry at room temperature .

3.4.2 Principle of Immunohistochemical assay

The immunohistochemical staining techniques is used for visualization of tissues antigens by reaction of a specific antibody (primary antibody) to its corresponding antigen (Anti-Elastin antibody [BA-4] ab9519) tissue sample , then a secondary antibody was added to the primary antibody and an enzyme complex with a chromogenic substrate, interposed by washing steps, the enzymatic activation of the chromogene results in a visible reaction product at the antigen site . the specimen counterstained and cover slipped, results are interpreted using light microscope (Kumar and Rudbck, 2009).

Table (3.2): The equipment used in envision technique ..

Equipments	Company
Microwave Oven	Clatronic, Europe
Micro Pipettes	Eppendrof, Germany
Positive Charge Slides	USA
Ph Meter	Fisher, UK
Vortex	Retsch, Germany
Timer	Japan
Slider Holder	Germany

Table (3.3) : The used reagents and chemical in envision technique .

Reagents	Company
Anti-Elastin antibody [BA-4] ab9519	abcam
Novocastra™ DAB substrate buffer	Novocastra™ DAB Laboratories Ltd, vision bio system TM Company, Newcastle, UK .
DAB 3,3-diaminobenzidine, liquid and substrate chromogen system	Dako, Denmark
Endogenous peroxidase, 3% Hydrogen peroxidise (H2O2)	Dako, Denmark
Ethanol	BDH, Poole, England
Haematoxylin	BDH, Poole, England
Phosphate buffer saline	Sigma, UK
Sodium citrate	BDH, Poole, England
Trypsin	BDH, Poole, England
Sodium Hydroxide	BDH, Poole, England
Calcium chloride	BDH, Poole, England

3.4.3 Reagents and Equipment

1-standard solvent used in immunohistochemistry .

2-50mM tris-buffered saline (TBS) pH (7.6).

3-Antigen retrieval solution (s) .

4- Enzyme retrieval solution (s) .

5-Antibody diluents .

6- Primary antibody .

7-Mounting medium .

8-Trypsin .

9-Calcium Chloride .

10- Sodium Hydroxide NaOH .

11-Sodium citrate

3.4.4 Staining Protocol

The novocastraTM polymer detection system (envision technique) using commercial kit from novocastra, Newcastle, UK, RE7150-K was used .

1-Sections were paraffinized into two changes of xylene of 4 minutes for each .

2-Rehydration: section were rehydrated by immersing the slides sequentially in graded alcohol solution 100% for 2 minutes, 95% for 1 minute, 70% for 1 minute and finally in PBS for 1 minute .

3-washed slides in running tap water.

4- In This Step Used Two Procedure For Antigen Retrieval .

- The Heat-Induced Epitope Retrieval or (HIER) used sodium citrate were performed by placing the slide in slide steel holder and it was immersed in 1 L of Tris – EDTA buffer pH 9, in large plastic container, the container was placed in microwave at (800W) for 25 minutes. After cooling the antigen retrieval solution for 15-30 minutes at (4 C°), the slides were removed and washed by PBS for 5 minutes.
- Proteolytic-Induced Epitope Retrieval or (PIER) used Trypsin, Cover sections with trypsin working solution.

Preparation of solution : Trypsin Working Solution (0.05%) :

Trypsin stock solution (0.5%) ----- 1 ml.

Calcium chloride stock solution 1% ----- 1 ml.

Distilled Water ----- 8 ml.

Adjust pH to 7.8 with 1N NaOH, and incubate for 10-20 minute then allow sections to cool at room temperature for 10 minutes. Rinse sections in PBS for 2x2 minutes.

5-Peroxidase Block solution was added for 5 minutes then washed in PBS for 2x5 minutes.

6-Protine Block solution was added for 5 minutes then washed in PBS for 2x5 minutes.

7-Tissue sections were incubated with diluted monoclonal primary antibody (1 : 100) for (Anti-Elastin antibody [BA-4] ab9519) for one hour at room temperature in appropriate dilution . Biopsies were washed in PBS 2x5 minutes.

8-Post primary block solution for 30 minutes ,washed in PBS 2x5 minutes.

9-Tissue section were incubated with a secondary antibody Novolink TM polymer mouse and rabbit immunoglobulins for 30 minutes, washed in PBS 2x5 minutes with gentle rocking .

10- DAB substrate buffer were used for 5-8 minutes, then washed with tap water .

11-Finally, biopsies were counterstained with hematoxylin, washed in water, dehydrated . After that, slides were mounted by 70% glycerol solution and use also DPX then covered by cover slip. The slides were examined by light microscope 10X, 40X and after comparing the tested sections with negative and positive control slides.

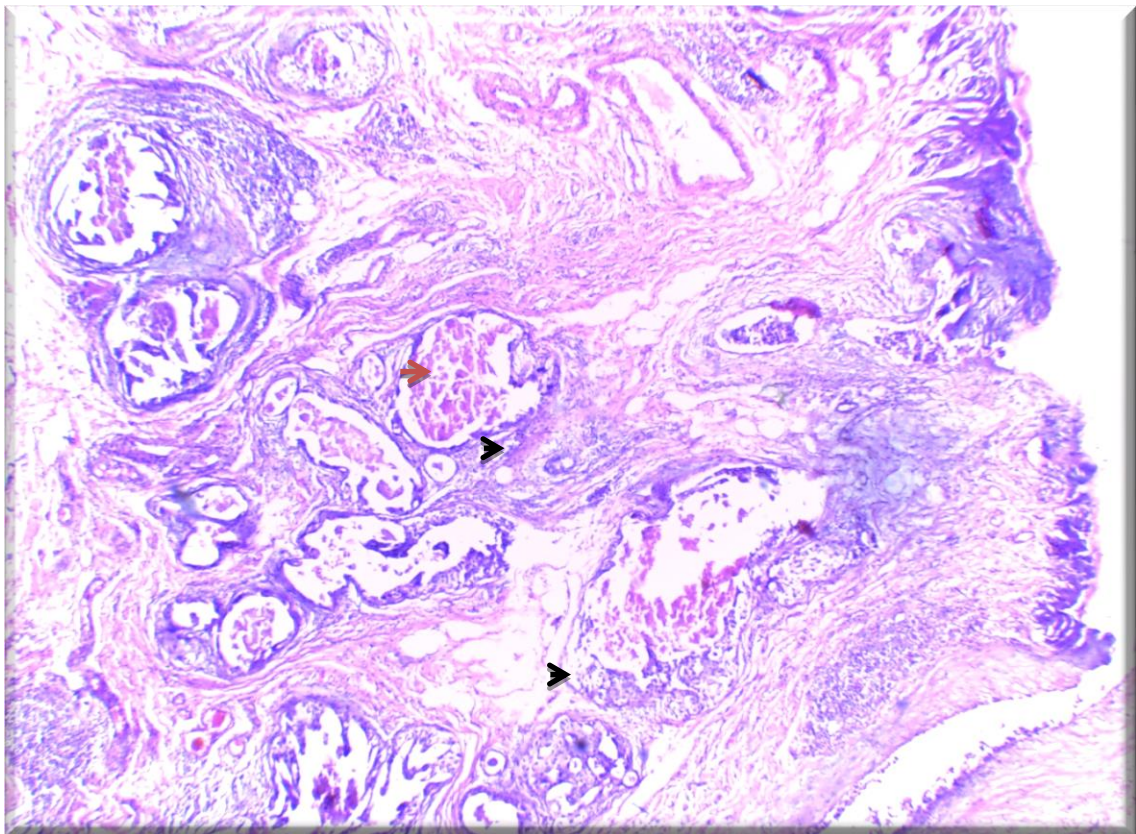
3.5 Statistical Analysis

Statistical analysis were performed by using the SPSS 2015 / correlation coefficient to show the correlation between Elastin protein expression with the ER, PR, Her2 expression and the age, The different was considered significant when the probability (p) value was $p \leq 0.01$,

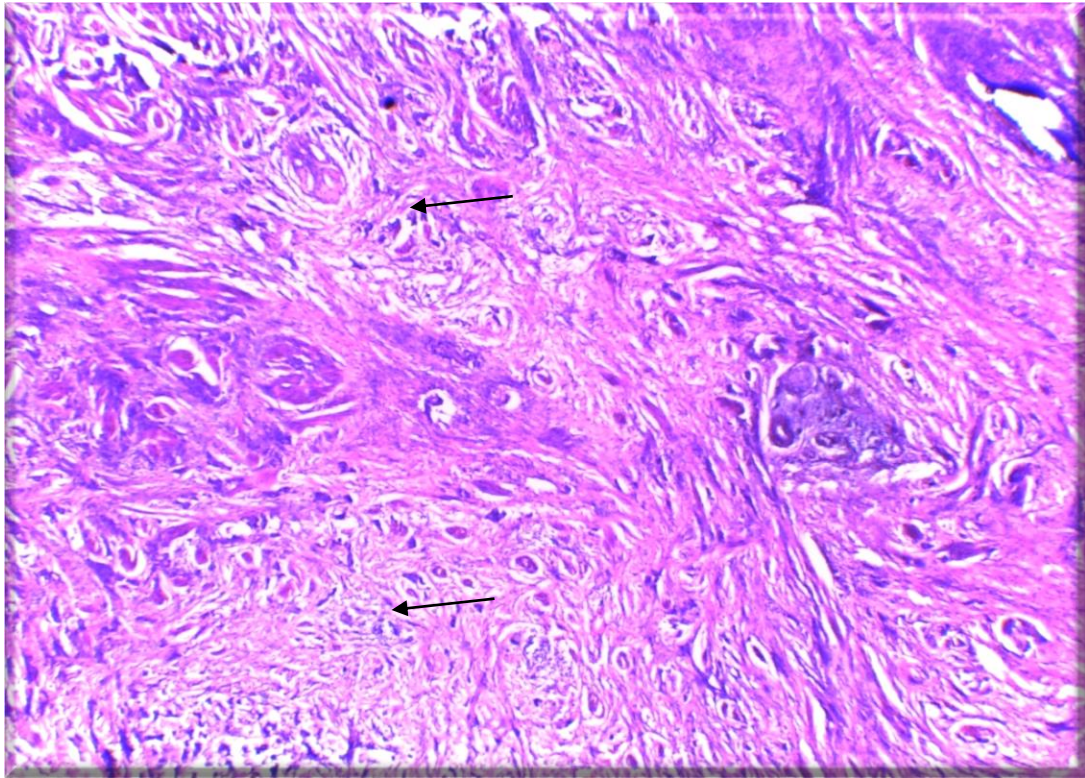
The Results

4.1 Histological study

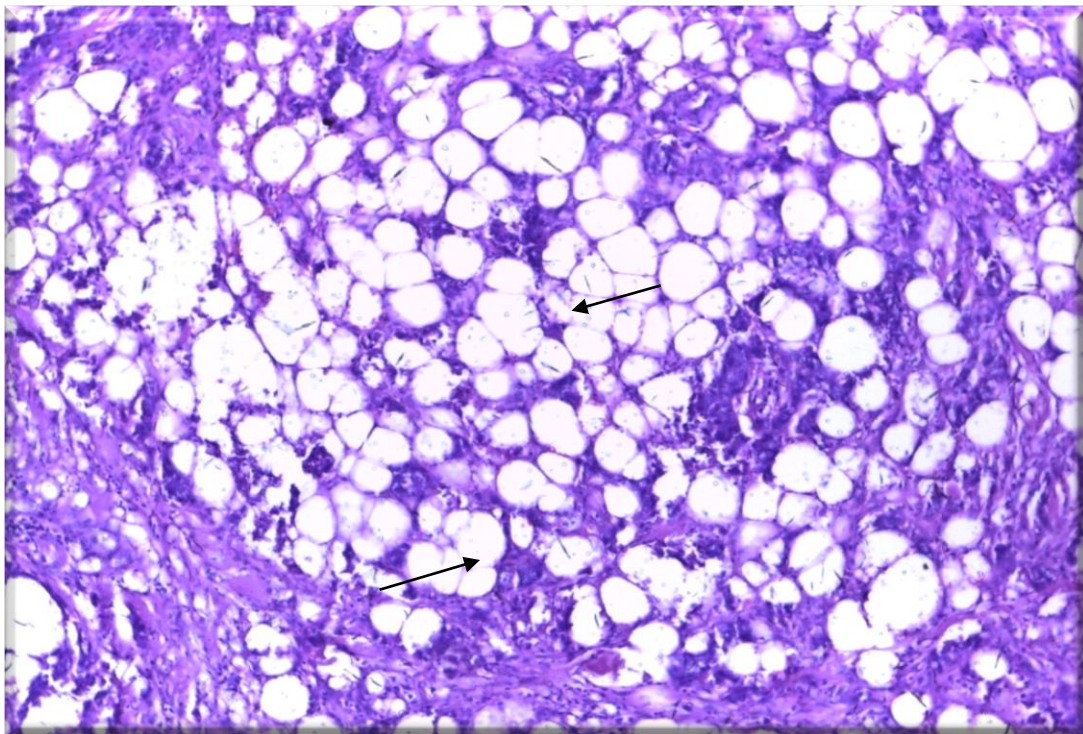
The sections were stained by Eosin and Heamatoxlin stains to investigate the architectural construction of the sections, the sections investigated as an invasive ductal carcinoma (IDC), the breast cancer cells break down the basement membrane and separated to the surrounding tissues (Fig.2,3 and 4).



Fig(4-2):Breast cancer cross section, invasive ductal carcinoma, shows the cancer cells invasive the surrounding tissue ➤, also in the duct ➡, H&E(10x).



Fig(4-3): Breast cancer cross section, invasive ductal carcinoma, shows the -cancer cells invasive the surrounding tissue (←), E & H(10x).

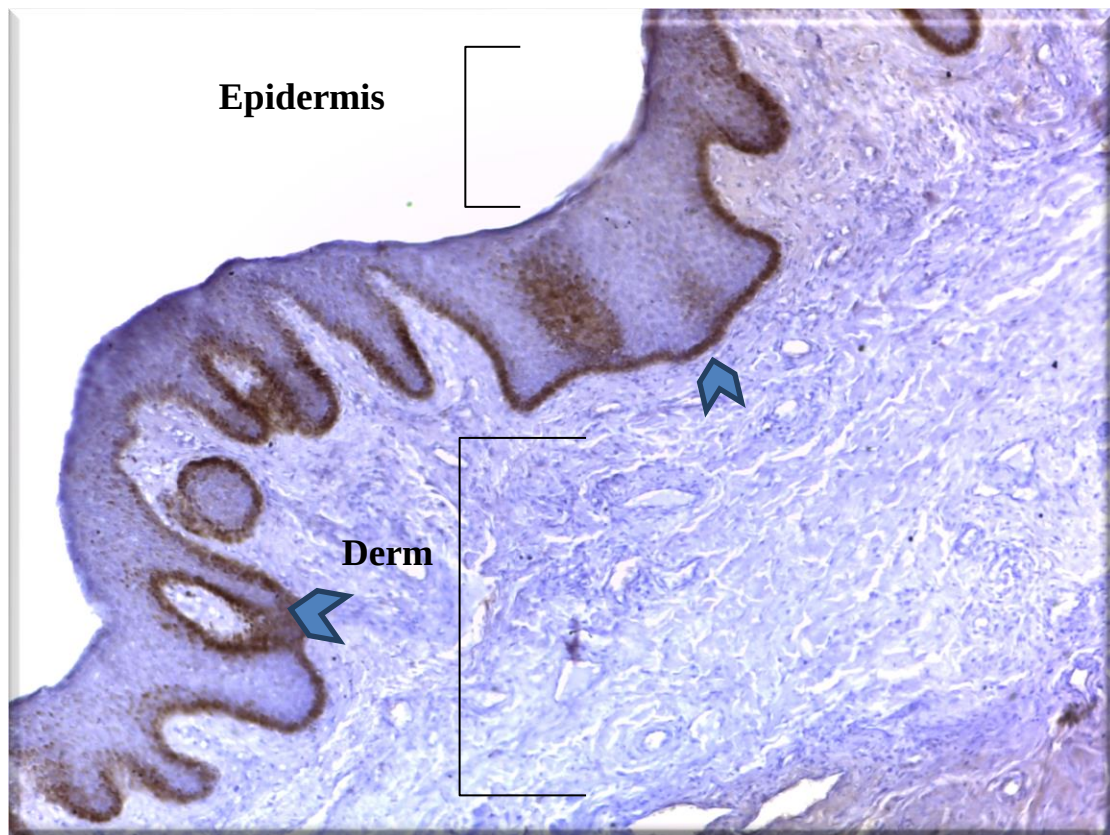


Fig(4-4): Breast cancer cross section, invasive ductal carcinoma, the-cancer invasive the surrounding the adipose tissue,(←) E & H(10x).

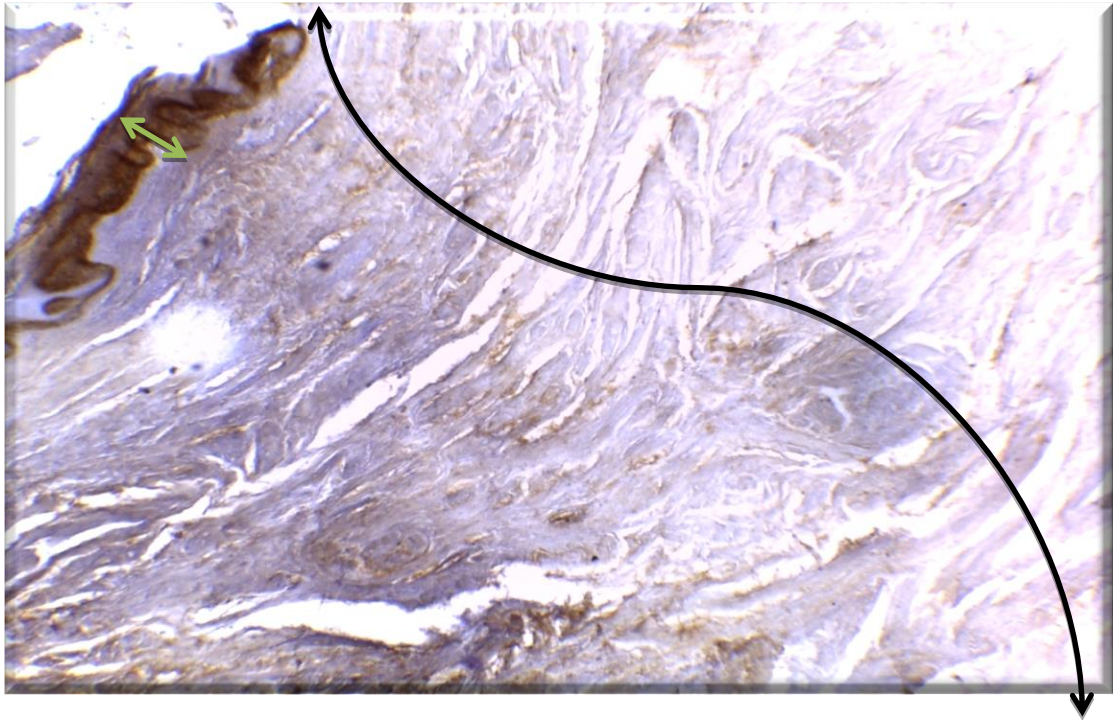
4.2 Immunohistochemical study

Section showed highly elastin expression in skin tissue as positive control. the protein deposited as brown or black in the epidermis and dermal basal is region, also in the subcutaneous (Fig.5,6 and 7).

While the negative control for elastin expression was obvious in section from skin stained without anti-elastin protein (Fig.8).



Fig(4-5): Cross section in skin showed positive expression of elastin in the epidermal layer of skin ➡ immunohistochemistry (4x).



Fig(4-6): Section in skin showed highly positive expression of elastin in epidermis and dermis (green double-headed arrow) immunohistochemistry (10x).

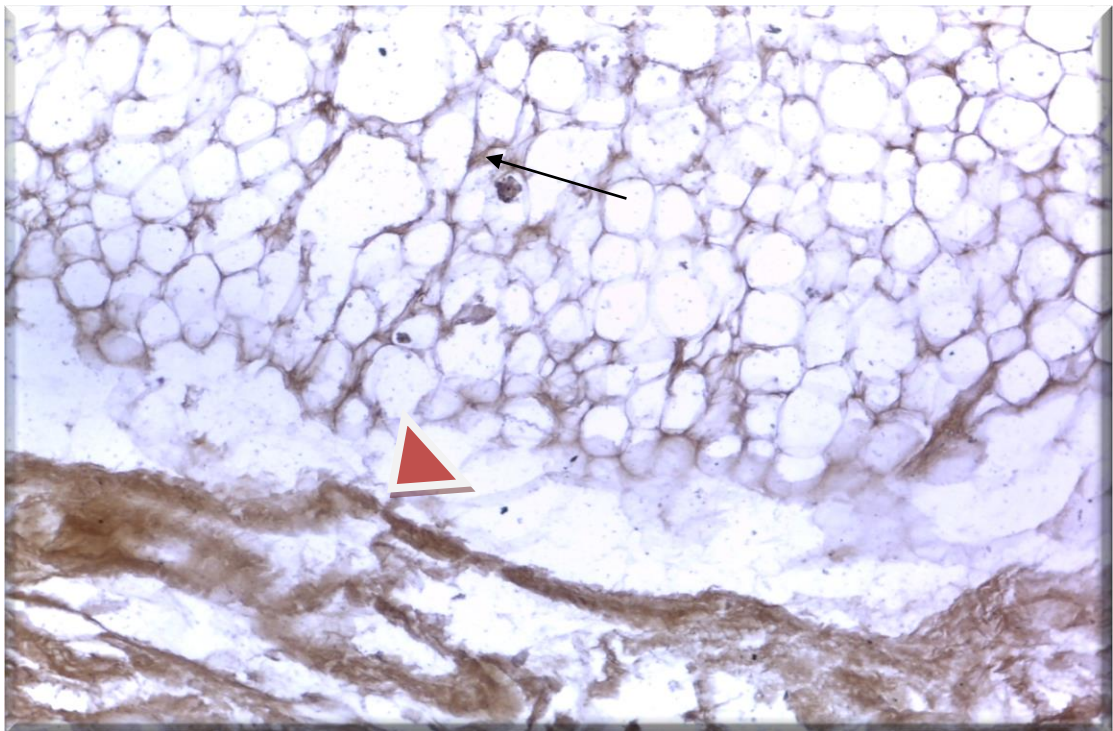
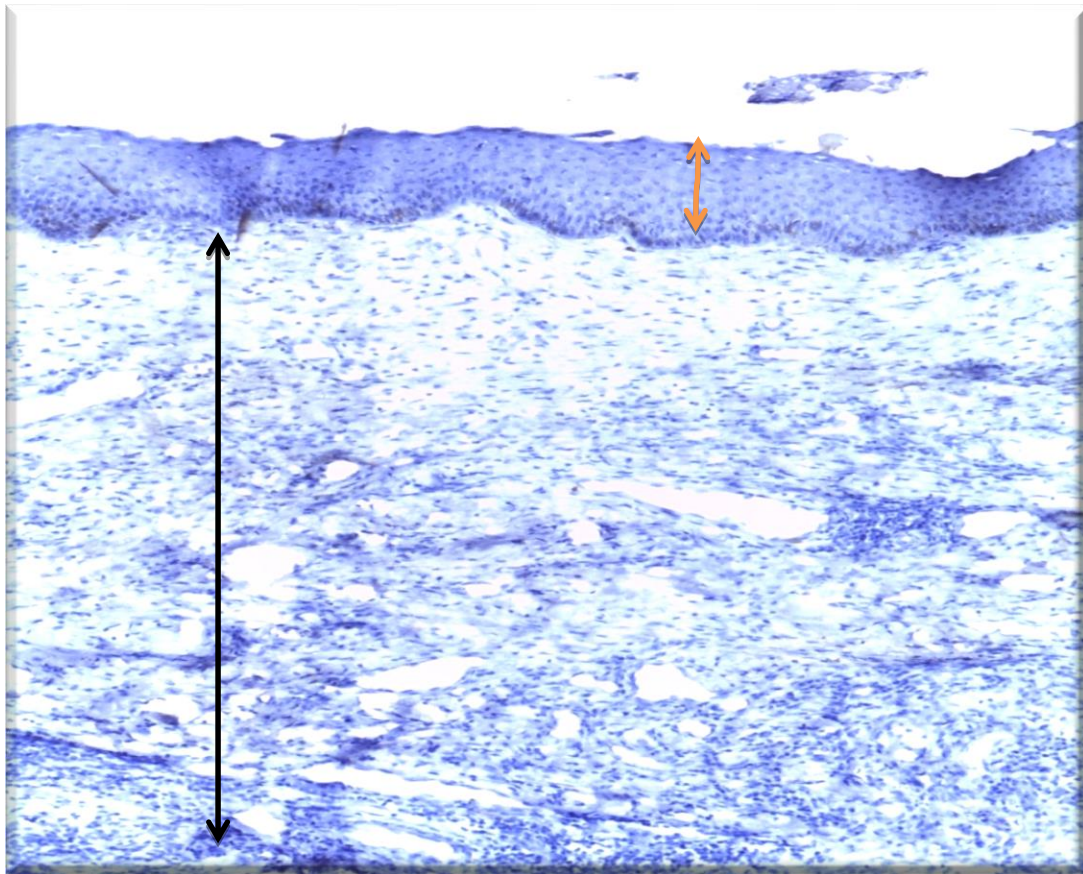


Fig (4-7): Section in skin showed highly positive expression of elastin in the subcutaneous layer (red triangle), adipose tissue in the skin tissue (black arrow) brown color) immunohistochemistry (10x).



Fig(4-8): The section showed normal epidermis  and normal wide dermis layer  . without-immun stain H&E stain (4x).

4.2 Relationship of age, grade. Stage and scores

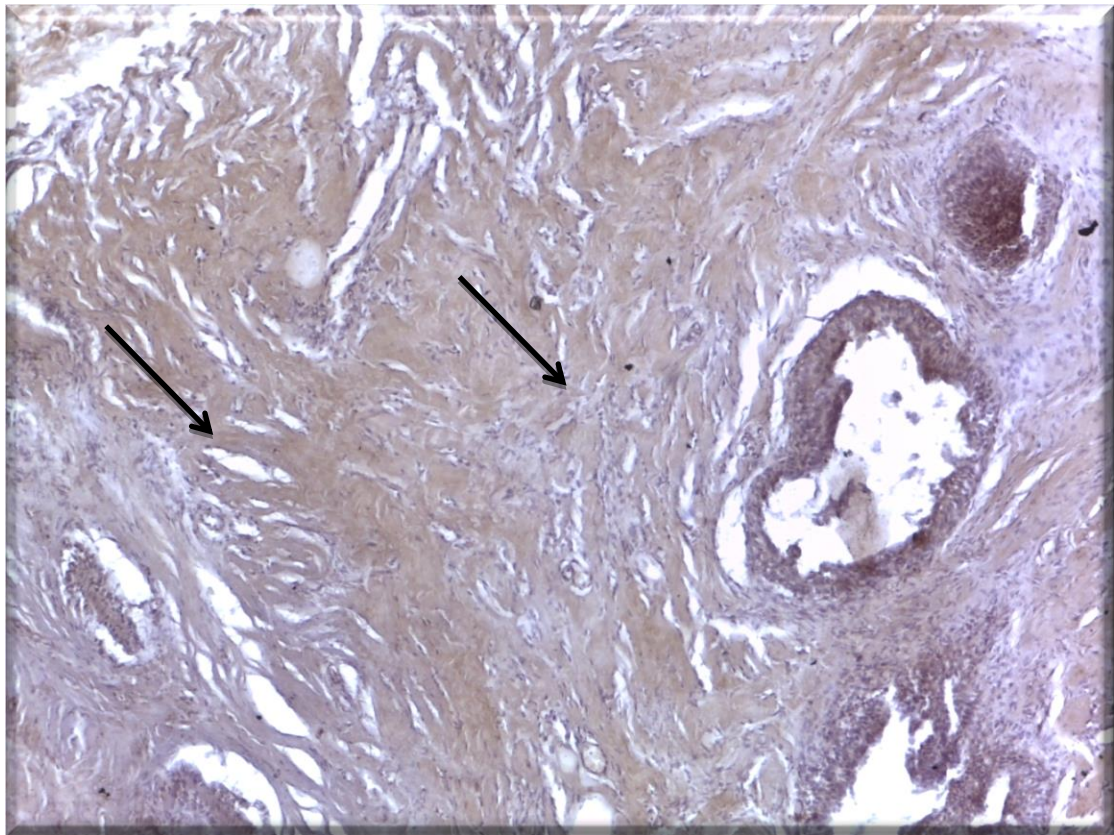
In this study the mean of the age of all 50 patients was 50 years, we have study the correlation between the elastin, ER, PR, and Her2 receptors protein expression with age, all the samples were in grade >2, stage> 3 and scores >3 which have been reported by the histopathologist ,Table (4-1), fig (18).

4.3 The correlation between elastin expression and age in (BC) patients

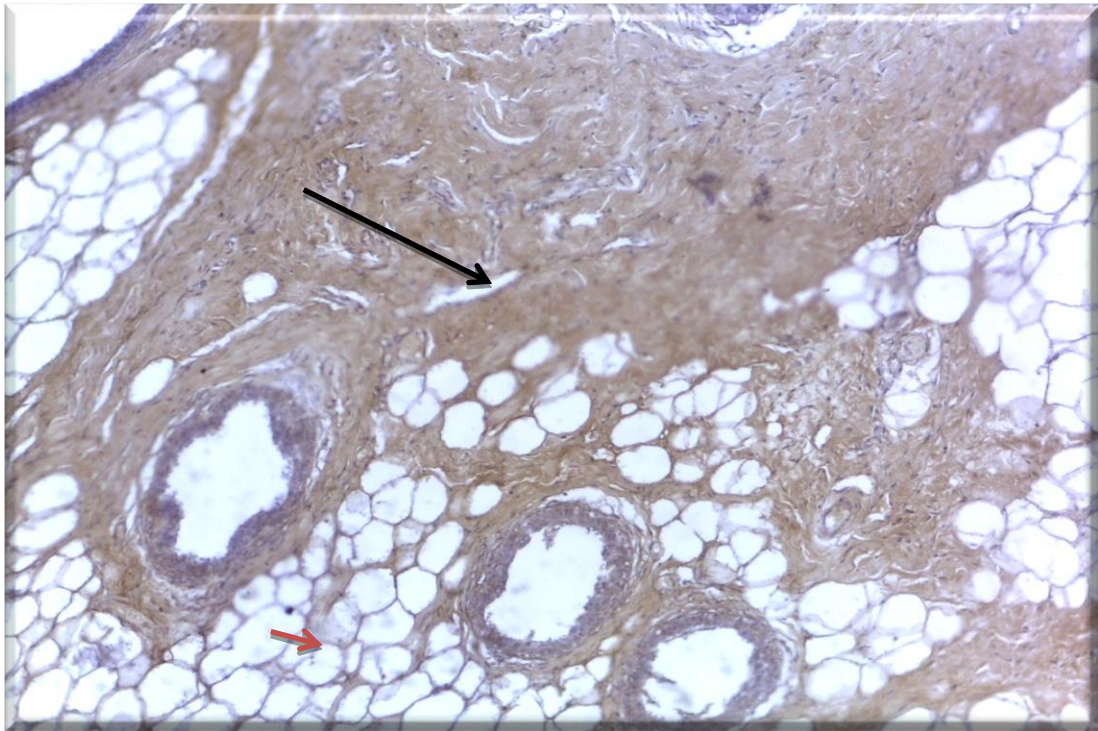
Recent study included 50 cases of breast carcinoma (women) , this study have been shown strong positive correlation the elastin protein expression and the age. there was positive significant correlation between

this factor and the age, the expression increase significantly with increasing the age in all (BC) patients, the value of correlation was (0. 45) with significant difference at $p < 0.05$ (Table 4- 1).

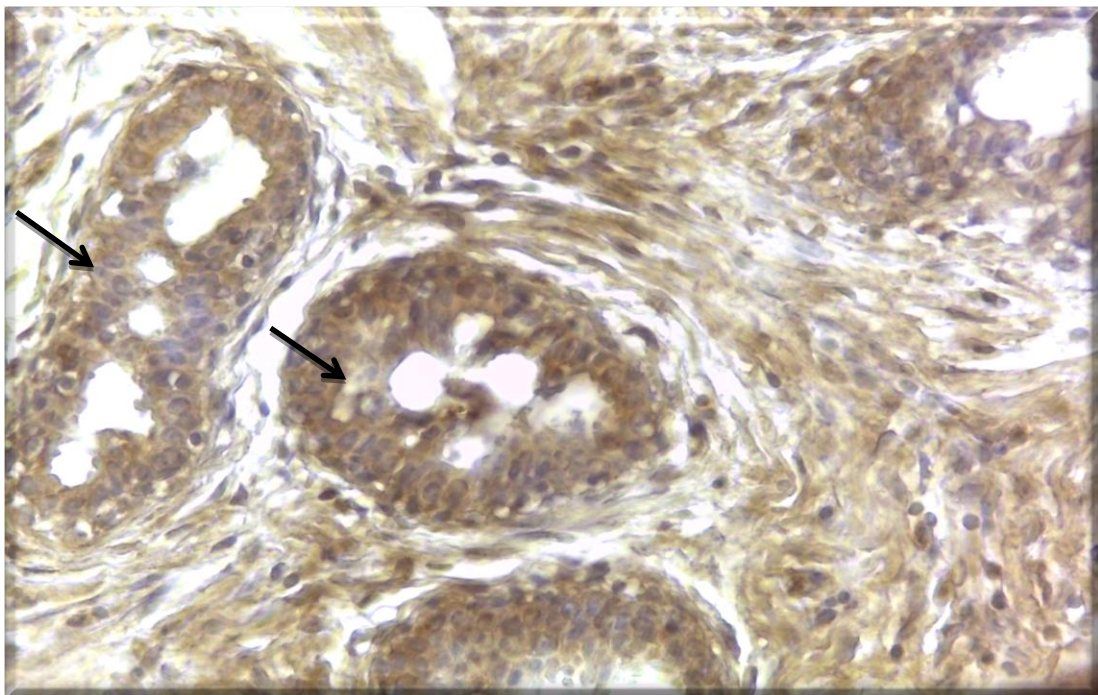
The histological investigation showed the elastin expression in the connective tissue around the ducts patient and between and surrounding adipose tissue , the microscopic investigation showed highly elastin expression around cancer cell inside the ducts (Fig 9,10 and 11)



Fig(4-9): Section in breast cancer tissue, elastin highly expression in woman with positive ER receptors, shows the elastin with brown color ().immunohistochemistry (10x).



Fig(4-10): Section in breast cancer tissue, elastin highly expression in positive ER receptors shows the elastin with brown color in the-surrounding connective tissue and adipose connective tissue immunohistochemistry (10x).



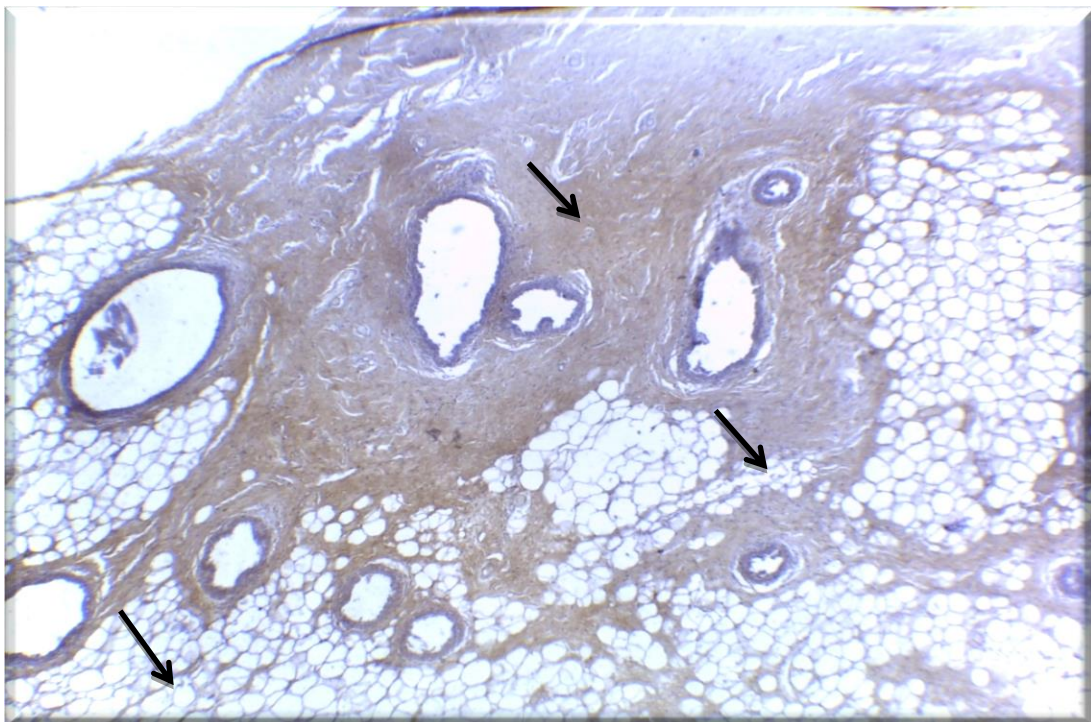
Fig(4-11): Section in breast cancer tissue, elastin highly expression in woman with positive ER receptors shows the elastin with brown color

surrounding the cancer cells in the duct ↘ . immunohistochemistry (10x).

Also this study has been shown positive significantly correlation between the elastin expression and the ER and PR positive expression 0.235 and 0.254 at the $p < 0.05$ (Table 4-1). This the results and data showed no correlation between the age and HER2 expression marker (Table 4-1).

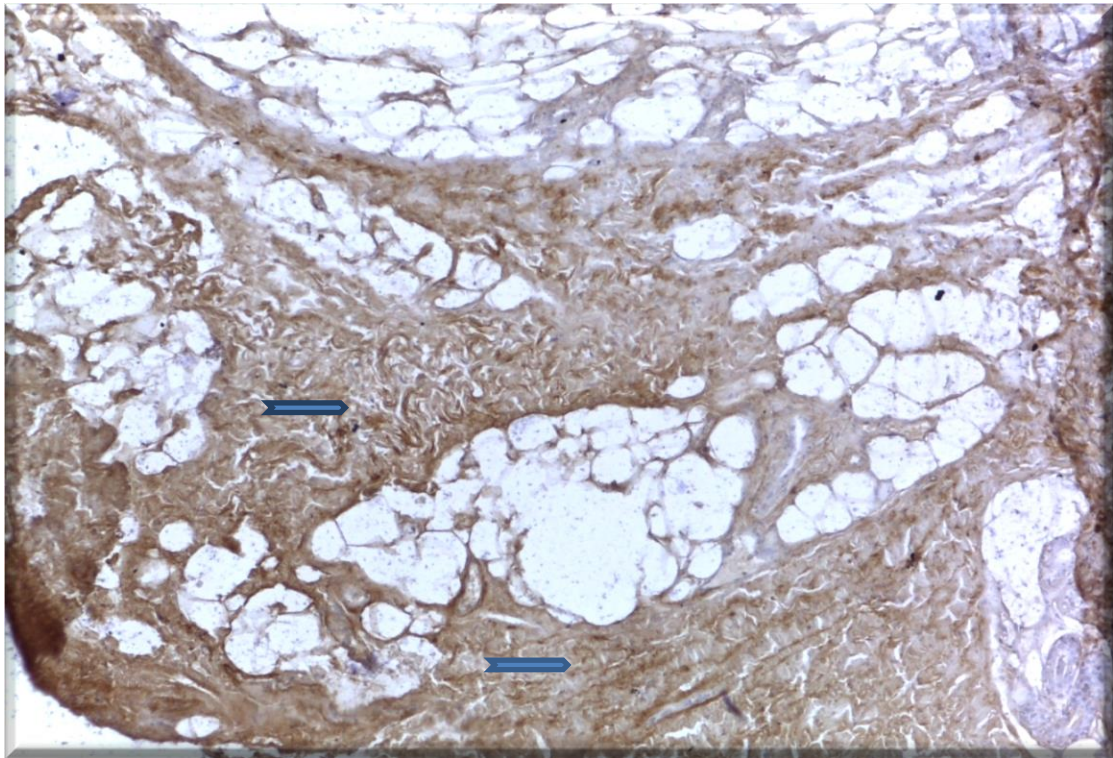
4.4 The correlation between elastin protein expression and ER and PR receptors

Our study showed there was significant positive correlation at $p < 0.05$ between the elastin expression and the positive ER and PR receptors in all specimens of (BC), it reached to expression, it was 0.312 and 0.355 respectively, Table (4- 1) , Fig (12 and 13).



Fig(4-12): Breast tissue, highly expression of elastin in woman with invasive ductal carcinoma has a positive PR receptor as brown

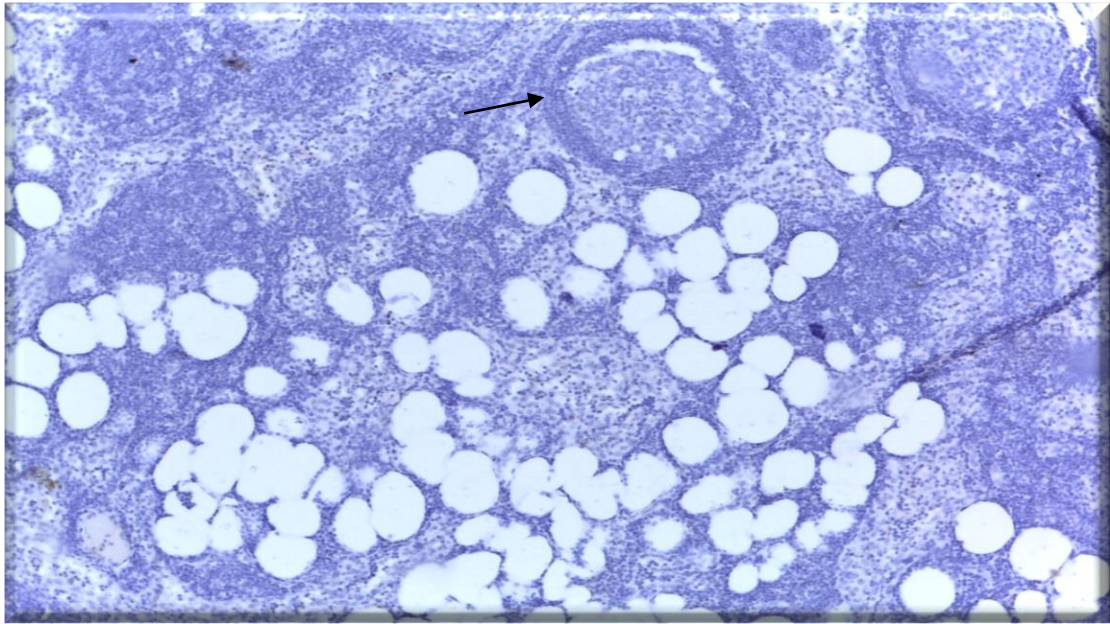
deposition ↘ immunohistochemistry (10x).



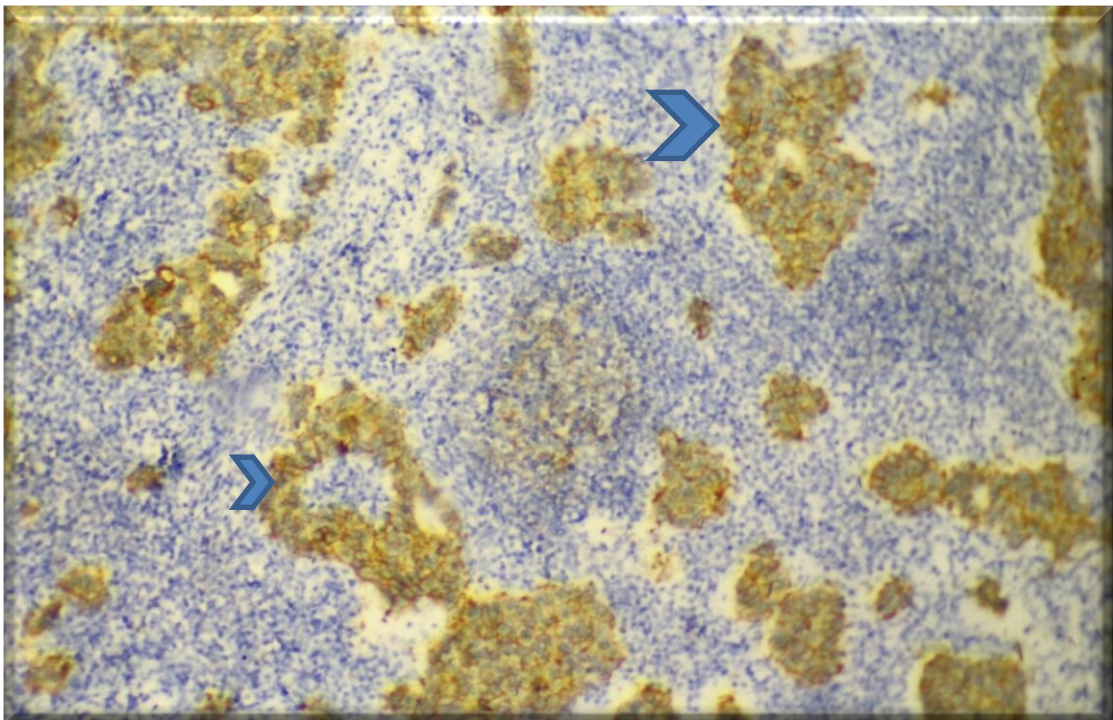
Fig(4-13): Breast tissue, highly expression of elastin in woman with invasive ductal carcinoma has a positive PR receptor as brown deposition around the adipose tissue ➡. immunohistochemistry H&E stain (4x).

4.5 The correlation between elastin protein expression and Her2

In the (BC) patients results showed that was negative correlation between the elastin protein expression, This study has shown there were negative correlation between the elastin protein expression and the Her2 positive marker, the value was (-0.214) , the-histopathology investigation haven't been shown any expression in sections with positive Her2 and negative ER and PR expression , Table (4-1),Fig (14 and 15).



Fig(4-14): Breast tissue, elastin negative expression in a woman with breast carcinoma has a positive Her2. Shows no brown color of elastin expression (—→) H&E stain (10x).



Fig(4- 15): Breast tissue, Her2 positive expression, brown color
 ➤ . imm unohistochemistry (10x).

4.6 The correlation between Her2, ER and PR receptors.

The results of this study have been shown there was negative correlation between the HER2 expression prognostic marker with ER and PR receptors, it was -0.299 and -0.038 respectively without significant difference in Table (4-1).

4.7The correlation between ER and PR prognostic parameter

This study showed significantly positive strong correlation between the expression of ER and PR prognostic parameters at the level $p < 0.01$. , the value of correlation was 0.541 as shown in table (4-1) Fig (16 and 17).

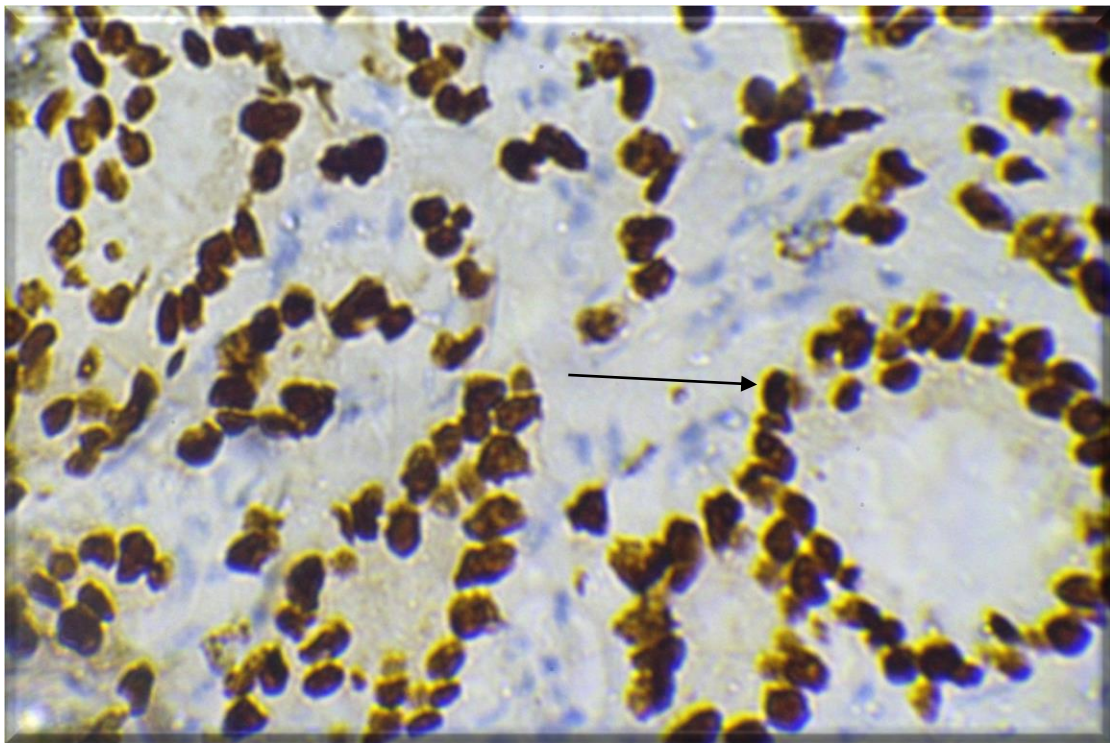


Fig (4-16): Section in breast cancer tissue, showed positive of ER receptors brown dots (), immunohistochemistry H&E stain (4x).

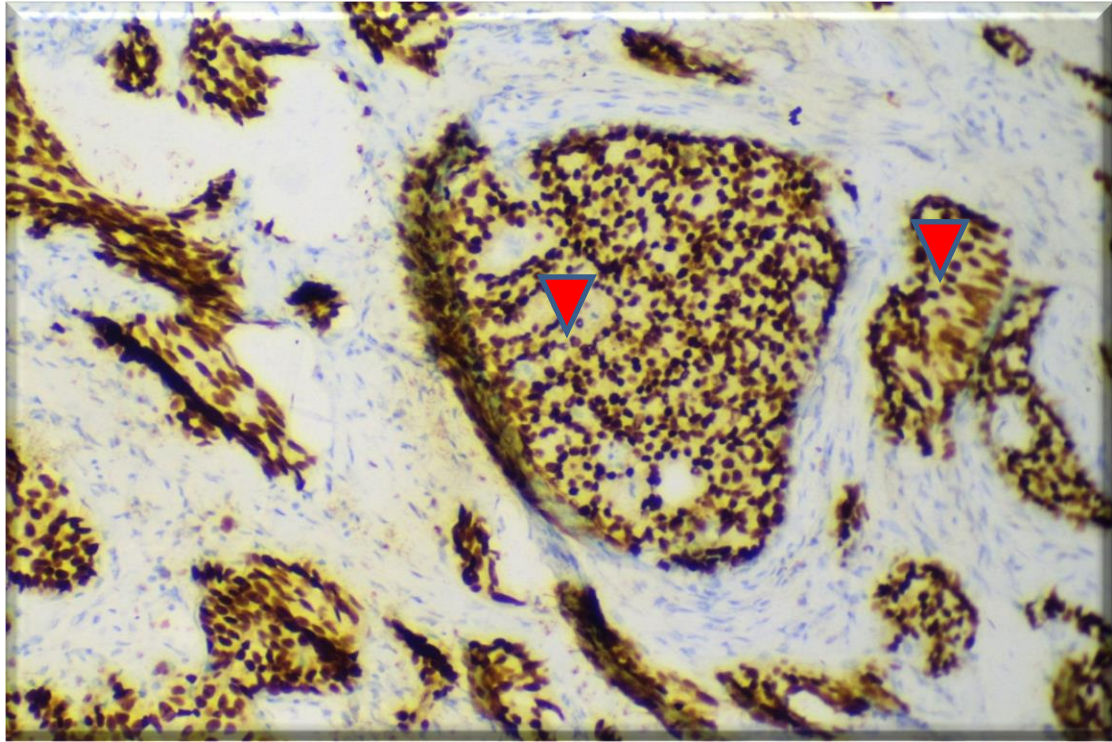


Fig (4-17): Breast tissue, PR positive expression.,  immunohistochemistry (4x).

Table (4-1): Correlation between the elastin expression with HER2, PR , ER and age.

	Elastin	HER2	PR	ER
Age	0.450**	- 0.247	0.235*	0.254*
Elastin		-0.214	0.312*	0.355*
HER2			-0.299	-0.038
PR				0.541**

**Correlation is significant at the 0.01 level.

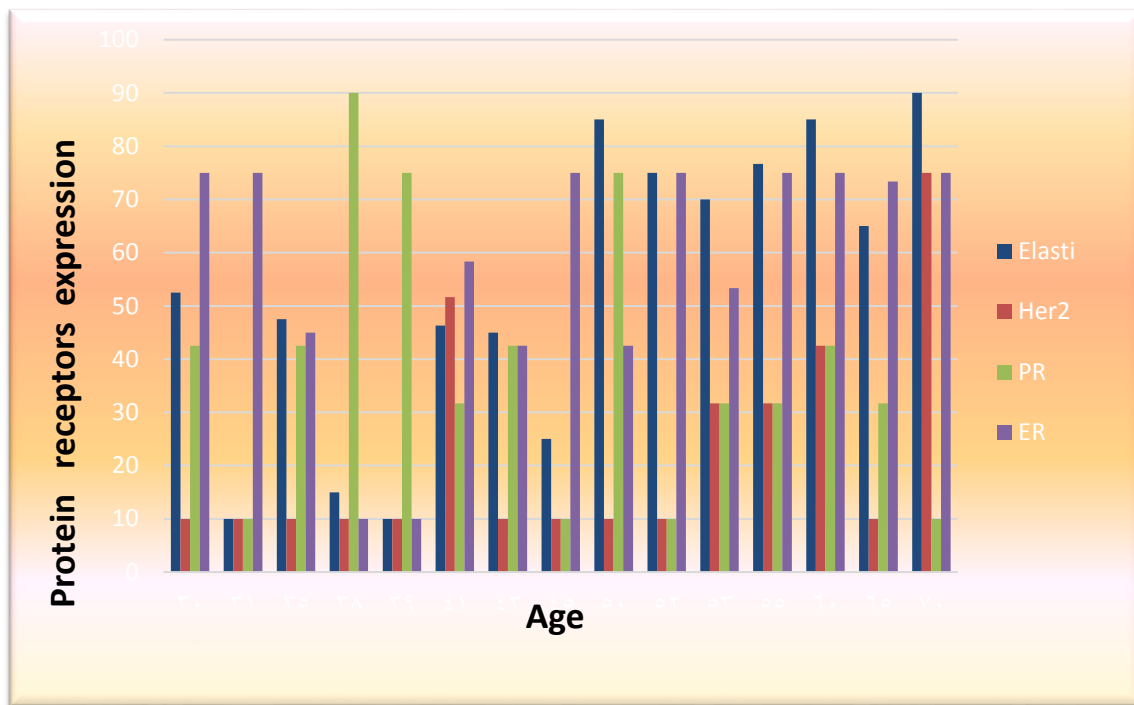


Fig (4-18): The histogram of the crude data between the protein receptors expression and the age.

Discussion

5.1 Positive and negative control

Elastin is an essential part of various human organ tissues such as skin, lung and arterie. the skin is the largest organ in the body, which is consist of two layers, Epidermis and dermis (Eroschenko, 2008).

The dermis consist of two major fibers proteins which are the collagen fibers and the elastin (Gartner *et al.*, 2007).

The elastin has been involved in many biological functions ,a restricted to elasticity is one of them (Delelle and Alix, 1999).

In current study , the control positive sections of skin had been stained by immune histochemistry kit by conjunction the primary anti elastin protein as shown in the result . while the negative one had been stain with free primary anti-body. The elastin produce by the fibroblast under the-effect of estrogen hormone, this fibers is associated with the level of estrogen and progesterone hormones (Stevenson and Thornton, 2007), previous studies discussed the effect of estrogen hormone on synthesis this type of fibers, the study of Sator *et al.*, (2001) had been discussed the effect of hormone replacement therapy (HRT) on increase epidermal skin elasticity and thickness. the study of Shah and Maibach (2001) found the epidermal thinning is associated with aging and maintain that the topical estradiol reduce epidermal thinning in aging skin and maintain the skin thickness.

There are a lot of changes in human skin are seen in post-menopausal women, there are numbers of studies documenting the differences seen following the menopause, also there a variation in skin thickness are seen during the menstrual cycle, the skin thickness was lowest at the start of the menstrual cycle, when the levels of estrogen and progesterone are

low, then increases in thickness with the rising levels of estrogen (Eisenbeiss, 1998).

5.2 Correlation between age and elastin expression

The first outcome of this study has been found, is a positive correlation between the age and elastin protein expression, in the normal condition there are negative correlation between the age and the elastic fibers synthesis, a lot of old and studies have been certain and agreed with this fact such as the study of (Pittet *et al.*, 2014 ; Cattell *et al.*, 1996).

The positive correlation between the elastin expression and the age, may be comes from the high levels of the sex hormones in these patients, the increase of hormones levels are come from effect of a lot of factors such as, intake contraceptive and the late menopause, these factors due to change in the levels of estrogen and progesterone hormone , also there were study found that the amount and the number of elastic fibers increase with the age of women who have children compare with another haven't children (Robert, 1999).

Many studies reported a sudden onset of skin aging several months after menopausal symptoms begin. The menopause causes hypo estrogenism, accelerating age-related deterioration, which results in thinner skin, an increase in number and depth of wrinkles, increased skin dryness, and decreased skin firmness and elasticity (Brincat, 2000) .

This study suggests that estrogen and progesterone level have an effect on elastin protein expression , the synthesis of elastin is depended on sex hormones levels, the study of Shikata *et al.*, (1979) were they found that the sex hormones and age had effected on elastin in the hip joint capsule of rats, they studied some metric parameters such as the-diameter of the elastic fiber and the ratio of the central elastin to the-peripheral microfibrils, they found it is greater in females than in males

after sexual maturation, also they had found the treatment with estrogen due to increase in the elastin fiber diameter and its content .

5.2.1 Correlation between age with ER , PR and HER2 expression

The another result of this study is positive association-correlation between the age and expression of ER and PR, in general incidence of breast cancer is correlates strongly with age, the highest rates of incidence being observed women with old ages , only six percentage occurring in women under forty years old (Hamadeh *et al.*, 2014). However, the (BC) usually more aggressive and advanced in the younger age groups (National Cancer Institute, 2009).

Our study is agreed with studies of Fatima *et al.*, (2005); Rosenberg *et al.*, (2006); Dunnwald *et al.*, (2007); Nisa *et al.*, (2008) have reported that the age has positive correlated with ER and PR with higher proportion of low grad and more differentiated carcinomas seen in older women, also it is has been agreed with a study of Survarchala (2011) which showed that the positive ER and PR receptors expression was increase of comparative with age , also agreed with study of Madhuri *et al.*, (2010) which has been showed that the negative ER and PR expression increase in the age between 40 and 60 years old compare with younger and woman more than sixty years . while it doesn't agreed with Gupta *et al.*, (2015) they didn't find any significant result between the age and the expression of ER and PR expression .

The possible explanation of this result might be comes from the fact that women with premenopausal have higher concentrations of circulating estrogen that would bind to cytosol receptors, which making them unavailable for routine immunohistochemistry assay. Previous studies Clark and Guire (1988), showed no difference in ER between

premenopausal and postmenopausal women of the same age. There are another factors could be effect on the ER, PR Positive or negative then it play a role in cell proliferation of (BC). previous studies reported that risk of ER+ PR+ (BC) is associated with null parity, a later age at first birth, a later age at menarche, and a higher body mass index.

A recent comparison study of age specific (BC) incidence rates by hormone receptor status led to hypothesize that hormone related factors associated with a lifestyle may be strongly related to positive ER and PR breast cancer than to other tumor subtypes, also these associations may vary by menopausal status (Yasui and Potter, 1999). additionally explored whether the heterogeneity among tumor subtypes varied by menopausal status (Huang *et al.*, 2000).

The correlation between the age and (BC) is variable among the communities, The breast cancer risk with increases age, in USA they estimated that five to seven percentage of breast carcinomas are presented in younger woman than 40 years old (Bland *et al.*,1998), while Iranian study of Pourzand *et al.*, (2011) had been shown it is about seventeen percentage.

5.3Correlation between the elastin with ER , PR and HER2 expression

The outcomes of this study have been shown there were a significant positive correlation between the expression of elastin protein expression and the expression of estrogen and progesterone receptors, this result agreed with Rasmussen *et al.*, (1985) when they had showed that the-increasing amount of elastin was related with increasing amount of ER While our study disagreed with it with another prognostic marker PR, They mention there are slightly correlated between elastin and progesterone receptors.

This result come as shown as in the previous discussion items from effect of the level sex hormone , as known, the elastin is the extracellular matrix protein and it is variable amounts depending on the tissue such as skin, lung and arteries (Partridge1970 ; Sage and Gray ;1980).

Estrogen receptors, positive and negative (BC) are emerging as distinct disease subtypes with different risk factors. accumulating evidence supports the need to evaluate (BC) etiology and risk separately for each of these tumor subtypes (Setiawan *et al.*, (2009);Chlebowski *et al.*, (2007); Cummings *et al.*, (2005); Colditz *et al.*, (2004).

The epidemiological high commended studies has reported strong associations between high levels of testosterone and estradiol and an increased risk of postmenopausal (BC), especially estrogen positive breast cancers (Key *et al.*, (2002); Missmer *et al.*,(2004); Zeleniuch-Jacquotte *et al.*, (2004);Cummings *et al.*, (2005);Kaaks *et al.*, (2005); Cummings *et al.*, (2009);Sieri *et al.*, (2009).

The study of Farhat *et al.*, (2011), had reveals that the ER-positive (BC) risk increased with higher testosterone levels , but this trend was not statistically significant after adjustment for estradiol, Also they found the estrogen positive cancer risk was approximately twofold higher in women with estradiol levels in quartiles 2–4 compared with women in quartile 1, independent of risk factors .

In another, Tworoger *et al.*, (2005) found the estradiol and free estradiol were statistically significantly positively associated with (BC) risk among women older than 60 years and among women with a body mass index of less than 25 kg/m².

Old study showed that estrogens and progesterone's may influence on synthesis of collagen and elastin (Kuhl ,1995), recently studies of Neubauer *et al.*, (2011) and Nallasamy (2017) Showed that the estrogen

and progesterone hormone have a role on Regulation of Collagen and elastic Fibers in pregnancy mice by increase the tissue stiffness of ECM.

In depended on the results of this study and additional to previous Old studies, the Prognosis of (BC) is related to a lot of variety of pathological markers such as ER and PR and HER2. Old studies such as studies of Shivas and Douglas (1972) had been showing that tumors with large amount of elastotic substance had better prognosis and there are a correlation between the elastosis and survival (Rasmussen *et al.*, 1985; Fisher *et al.*, 1993) .

In this study we found a positive coloration between the expression of elastin protein expression and positive status of ER and PR receptors , which be consider as a good prognostic markers indicator specially when it associated with mammography screening detection the elastosis.

A new study of Chen *et al.*, (2014) has been certain there was a strong correlation between the elastosis and stellated shap of tumors, they found there are a correlation between the elastosis and the positive ER and low cell proliferation, also study of Youk *et al.*, (2013) had shown the correlated between the mean elasticity value and the estrogen receptor and progesterone receptor .

5.4Correlation between HER2 with ER and PR expression

A negative correlation between the Her2 expression and both of expression of progesterone and estrogen receptors.

This result agreed with a study of Pourzand *et al.*, (2011) who found negative correlation between the negative progestron receptor expression and the Her2 over expression. This result could be comes from the low percentage of Her2 over-expressed in about 15-20% of (BC) cases .

5.5 Correlation between ER and PR expression

The last outcome of this study have shown a positive correlation between ER and PR expression. ours result agreed with Allred (2010), who had said there are highly correlated expression between ER and PR receptors.

The determination of some status of important receptors such as Progesterone –receptor and Estrogen status has become a standard practice in the treatment of (BC).

Estrogen expression was demonstrated in 80-90% of (BC) cases (Grann *et al.*, 2005) , while progesterone expression was demonstrated in 70-80% of cases (Esteva and Hortobagyi, 2004).

Some studies had shown a variation in steroid receptors status between different population also there were differences depend on the race ,particularly between black and white (Suvarchala and Nageswararo, 2011). Study of Gupta *et al.*, (2015) reveals that the Indian females compare with American women with breast cancer have low hormone receptors positive rate.

Conclusions

The results concluded :

- 1.Strong association with a positive significant correlation between ER+ and PR+ and Her2- with elastin protein expression .
- 2.Findings significant correlation between ER and PR positive status On the other hand, These findings showed the importance of these biomarkers because they provide valuable prognostic information for best therapeutic decision.
- 3.Results the heavy expression of elastosis and mammography detection of breast cancer.
- 4.The level of elastin associated with low-grade tumors and good prognosis.

Recommendations

The study recommended :

- 1.Diagnosis the subtype of elastin in relation to (BC) and identified the-molecular basis of it.
- 2.Genotype Study to correlate the elastin expression with the most gens associated with (BC) in patients.

References

- ❖ **Allred, D.C.2010.** Issues and updates: evaluating estrogen receptor-alpha, progesterone receptor, and HER2 in breast cancer *Mod Pathol* , vol. 23 Suppl 2 (pg. S52-S59).
- ❖ **Almine, J.F.; Bax, D.V.;Mithieux, S.M.;Nivison-Smith, L.;Rnjak, J.;Waterhouse, A.;Wise, S.G. and Weiss, A.S. 2010.** Elastin-Based Materials. *Chem Soc Rev*39, 3371-3379
- ❖ **Althuis, M. D.; Brogan, D. R.; Coates, R. J.; Daling, J. R.; Gammon, M. D.; Malone, K. E.; Schoenberg, J. B.; et al. 2003.** Hormonal Content And Potency Of Oral Contraceptives And Breast Cancer Risk Among Young Women. *British Journal Of Cancer*, 88, 50-57
- ❖ **American cancer society (ACS). 2012.** Cancer facts & Figure 2012.
- ❖ **American Cancer Society (ACS). 2013.**Detailed Guide: Breast cancer.Cancer.org.Accessedat<http://www.cancer.org/acs/groups/cid/documents/webcontent/003090-pdf.pdf>.
- ❖ **Axelrod, D.; Smith, J.; Kornreich, D.; Grinstead, E.; Singh,B.; and Cangiarella, J. 2008.** Breast cancer in young women. *Journal of American College Surgeons*, 206, 1193–1203.
- ❖ **Azzopardi, J.G.; and Laurini, R.N. 1974.** Elastosis in breast cancer. *Cancer.* ;33:174–183. doi: 10.1002/1097-0142(197401)33:1<174::AID-CNCR2820330126>3.0.CO;2-X.
- ❖ **Baglietto, L.; English, D. R.; Gertig, D. M.; Hopper, J. L.; and Giles, G. G. 2005.** Does Dietary Folate Intake Modify Effect Of Alcohol Consumption On Breast Cancer Risk? Prospective Cohort Study. *BMJ*, 331, 807 .
- ❖ **Barnes, D.M.; Hanby, A.M.;. 2001.** Oestrogen and progesterone receptors in breast cancer: past, present and future. *Histopathology*, 3, 271-4.
- ❖ **Bax, D. V.; Rodgers, U. R.; Bilek, M. M and Weiss A. S. 2009.** Cell adhesion to tropoelastin is mediated via the C-terminal

GRKRRK motif and integrin $\alpha V\beta 3$. *J. Biol. Chem.* 284 28616–28623.

- ❖ **Bergamaschi, A.; Tagliabue, E.; Sorlie, T.; Naume, B.; Triulzi, T.; Orlandi, R.; Russnes, H.G.; Nesland, J.M.; Tammi, R.; and Auvinen, P. 2008.** Extracellular matrix signature identifies breast cancer subgroups with different clinical outcome. *J. Pathol.* 214, (3) 357–367.
- ❖ Bissell, M.J; and Radisky, D.2001. Putting Tumours In Context. *Nat Rev Cancer*,1:46–54.
- ❖ **Bland, K.I.; Menk, H.R.; Scott-Cancer, C.E.; Morrow, M.; Winchester, D.J.; and Winchester D.P.1998 .** The National Cancer Data Base 10-Year Survey Of Breast Carcinoma Treatment At Hospitals In The United State *Cancer*,83: 1262-73.
- ❖ **Boyd, N.F.;Dite, G.S., et al. 2002.** Heritability of mammographic
- ❖ **Bray, F.; Ren, J.S. ; Masuyer, E. and Ferlay, J. 2012.** Global estimates of cancer prevalence for 27 sites in the adult population in 2008. *Int. J. Cancer*, 132: 1133-1145.
- Breast Cancer. *Lancet Oncol*, (2; 3), 133-40.
- ❖ **Brincat, M.P. 2000.** Hormone replacement therapy and the skin. cancer. *Epidemiol Rev*, (15; 1), 110-32.
- ❖ **Cancer. Epidemiol Rev. 1993.;**15:36-47.
- ❖ **Cattell, M.A.; Anderson, J.C. and Hasleton P.S.1996.** Age-related changes in amounts and concentrations of collagen and elastin in normotensive human thoracic aorta.*Clin Chim Acta*.9;245(1):73-84.
- ❖ **Chen, Y.; Klingen, T.A.; Wik, E.; Aas, H.;Vigeland, E.; Liestøl, K.; Garred, Ø.; Mæhlen, J.; Akslen, L.A.; Lømo, J.2014.** Breast cancer stromal elastosis is associated with mammography screening detection, low Ki67 expression and favourable prognosis in a population-based study. **Diagn Pathol.** 2014 Dec 19;9:230. doi: 10.1186/s13000-014-0230-8.

- ❖ **Chlebowski RT.; Anderson GL.; Lane DS.; et al. 2007.** Predicting Risk Of Breast Cancer In Postmenopausal Women By Hormone Receptor Status. J Natl Cancer Inst;99(22):1695–1705.
- ❖ **Clark, G.M.; and Guire ,W.L.M.c .1988.** Steroid receptors and other prognostic factors in primary breast cancer. Semin Oncol 15: 20–25.
- ❖ **Clavel-Chapelon, F.,& Group, E. N. E. 2002.**Differential Effects Of Reproductive Factors On The Risk Of Pre- And Postmenopausal Breast Cancer. Results From A Large Cohort Of French Women. British Journal Of Cancer, 86, 723-727.
- ❖ **Colditz, G.A.; Rosner, B.A.; Chen, W.Y.; Holmes, M.D.; and Hankinson, S.E.2004.** Risk Factors For Breast Cancer According To Estrogen And Progesterone Receptor Status. J Natl Cancer Inst;96(3):218–228.
- ❖ **Collaborative Group on Hormonal Factors in Breast Cancer .2002.** Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease. Lancet, Jul 20; 360 (9328):187-195.
- ❖ **Cummings S.R.; Lee J.S.; Lui L.Y.; et al. 2005.** Sex Hormones, Risk Factors, And Risk Of Estrogen Receptor-Positive Breast Cancer In Older Women: A Long-Term Prospective Study. Cancer Epidemiol Biomarkers Prev. 2005;14(5):1047–1051.
- ❖ **Cummings S.R.; Tice J.A.; Bauer S.; et al.2009.** Prevention Of Breast Cancer In Postmenopausal Women: Approaches To Estimating And Reducing Risk. J Natl Cancer Inst ;101(6):384–398.

- ❖ **Debelle, L And Alix, A. J. P .1999.** The Structure Of Elastin And Their Function. *Biochimie* . 81,981-994. density, a risk factor for breast cancer. *N Engl J Med*, (347; 12) 886-94 .
- ❖ **Doucet, S. *et al* .2009.** Hausberger, Martine, ed. "The Secretion of Areolar (Montgomery's) Glands from Lactating Women Elicits Selective, Unconditional Responses in Neonates". *PLoS ONE*. 4 (10): e7579.
- ❖ **Drake, R.; Vogl, A. and Mitchell, A. 2009.** Gray's Anatomy For Students (2nd Ed.). Retrieved From [Http://Books.Google.Com](http://books.google.com) .
- ❖ **Drake, Richard, L.; Vogl,; Wayn,; Tibbitts,; Adam, W.M.; and Mitchell .2005.** Gray's anatomy for students. illustrations by Richard Richardson, Paul. Philadelphia: Elsevier/Churchill Livingstone. [ISBN 978-0-8089-2306-0](#).
- ❖ **Dunnwald, L.K.; and Rossing, M.A.Li.Ci. 2007.** Hormone Receptor Status, Tumor Characteristics, And Prognosis: A Prospective Cohort Of Breast Cancer Patients. *Breast Cancer Res*;9(1):1-10.
- ❖ **Eisenbeiss, C.; Welzel, J.; and Schmeller ,W.1998.**The influence of female sex hormones on skin thickness: evaluation using 20 MHz sonography. *Br J Dermatol* .;139:462–7essential for elastic fibre development in vivo. *Nature*, 415, 168–171.-=
- ❖ **Eroschenko, V.P. 2008.**Difiore's Atlas Of Histology Withbfunctional Correlation . Lippincott Williams & Wilkins.11th.
- ❖ **Esteva,F.J.; and Hortobagyi,G.N.2004.** Prognostic Molecular Markers In Early Breast Cancer. *Breast Cancer Res*; 6: 109- 18.
Estrogen Receptor And Progesterone Receptor Status. *Am J Epidemiol* ;151(7):703–14.
- ❖ **Fact Sheet No.297:5.**
- ❖ **Farhat,G.N.; Cummings,S.R.; Chlebowski,R.T.; Parimi,N.; Cauley, J.A. ; Rohan,T.H.; Huang,A.J.;Vitolins,M.; Allan Hubbell,F. ; Manson,J.E.; Cochrane,B.B.; Lane, D.S.; and Lee, J.S.2011.**Sex Hormone Levels And Risks Of Estrogen

Receptor–Negative And Estrogen Receptor–Positive Breast Cancers .J Natl Cancer Inst. Apr 6;103(7):562-70.

- ❖ **Fatima, S.; Faridi, N. and Gill S.2005.** Breast Cancer: Steroid Receptors And Other Prognostic Indicator. J Coll Physicians Surg Pak ;15(4):230-3.
- ❖ **Fayed, L.2009.** Types Of Breast Cancer. About.Com: Cancer. Jul 09,[20Mar. 2010].[Http://Cancer.About.Com/Od/Breastcancer/A/Cancertypes.Html](http://Cancer.About.Com/Od/Breastcancer/A/Cancertypes.Html)
- ❖ **Ferlay, J.; Shin, H.R.; Bray, F. and *et al.* 2010.** globocan 2008, Estimates Of Worldwide Burden Of Cancer In 2008 Cancer Muzahem MY Al-Hashimi And Xingjian Wang Incidence And Mortality Worldwide: IARC Cancer Base; No.10. 127, 2893-917.
- ❖ **Fisher, E.R.; Anderson, S.; Redmond,C.; and Fisher, B.1993.** Pathologic findings from the National Surgical Adjuvant Breast Project protocol B-06. 10-year pathologic and clinical prognostic discriminants. Cancer.
- ❖ **Fisher, ER.; Redmond, CK.Liu. H.; Rockette, H. and Fisher B.1980.** Correlation Of Estrogen Receptorand Pathogenic Characteristics Of Invasive Breast Cancer. Cancer ;45:349-53.
- ❖ **Frantz ,C.; Stewart, K.M. and Weaver V.M.2010.** The Extracellular Matrix At A Glance.J Cell Sci, 123:4195–4200.
- ❖ **Gao, Y. T.; Shu, X. O.; Dai, Q.; Potter, J. D.; Brinton, L. A.; Wen, W.; and Sellers, T. A.; *et al.* 2000.** Association Of Menstrual And Reproductive Factors With Breast Cancer Risk: Results From The Shanghai Breast Cancer Study. International Journal Of Cancer, 87, 295-300.
- ❖ **Gartner, L. P.; Hiatt, J. L.; and Strum, J. M.2007.** Cell Biology And Histology.Lippincott Williams & Wilkins.6th Edition.

- ❖ **Gefen, A. and Dilmoney, B. 2007.** Mechanics Of The Normal Woman's Breast. Technology And Health Care, 15, 259–271.
Retrieved From [Http://iospress.Metapress.Com/Index/W36324241468483q.Pdf](http://iospress.metapress.com/index/W36324241468483q.pdf)
- ❖ **Glaubitz, L.C.; Bowen,J.H. C.o.x.E.B. .; and McCarty, K.S. J.r .1984.**Elastosis in human breast cancer. Correlation with sex steroid receptors and comparison with clinical outcome.Archives of Pathology & Laboratory Medicine 01, 108(1):27-30
- ❖ **Globocan. 2008.**Cancer Incidence And Mortality Worldwide. Lyon, France: International Agency For Research On Cancer; 2010.
- ❖ **Gown, A.M. 2008.** Current Issues In ER And HER2 Testing By IHC In Breast Cancer. Mod Pathol. 21, S8-S15.
- ❖ **Grann,V.R.;Troxel,A.B.;Zojwalla,N.J.;Jacobson,J.S.;Hershman,D and Neugut AI. 2005.**Hormone Receptor Status And Survival In A Population- Based Cohort Of Patients With Breast Carcinoma. Cancer, 103(11): 2241- 51
- ❖ **Gupta, D.; Gupta,V.; Marwah, N.; Gill, M.; Gupta, S.d Gopal,G.; Jain, P.;and Sen,R.2015 .** Correlation Of Hormone Receptor Expression With Histologic Parameters In Benign And Malignant Breast Tumors. 10.
- ❖ **Hamadeh, R.R.; Abulfatih, N.M.; Fekri,M.A.and Al-Mehza H.A.2014.** Epidemiology Of Breast Cancer Among Bahraini Women: Data From The Bahrain Cancer Registry. Sultan Qaboosuniv Med J ; 14: 176-182.
Hand Book Of Cancer Prevention. IARC Press, Vol 6.
- ❖ **Haslam, S.Z .1990.**Stromal-Epithelial Interactions In Normal And Neoplastic Mammary Gland Cancer Treat Res 53:401-420.
- ❖ **Holmberg, E.; Anderson, H.; Lundell, M.and Karlsson, P. 2005.** The Impact Of Reproductive Factors On Breast Cancer Risk-
-The Feasibility Of Using Swedish Population-Based Registers To

Account For The Effect Of Confounding In Cohort Studies. *Cancer Causes & Control*, 16, 235-243.

- ❖ **Hornebeck, W.; Tixier, J. M.; and Robert, L. 1986.** Inducible adhesion of mesenchymal cells to elastic fibers: elastinectin. *Proc. Natl. Acad. Sci. U.S.A.* 83 5517–5520. 10.1073/pnas.83.15.5517
http://seer.cancer.gov/archive/csr/1975_2009_pops09/results_merged/sect_04_breast.pdf
- ❖ **Huang, W.Y.; Newman, B.; Millikan, R.C.; Schell, M.J.; Hulka, B.S. and Moorman P.G. 2000.** Hormone – Related Factors And Risk Of Breast Cancer In Relation To
- ❖ **Hughes, C.C. .2008.** Endothelial-Stromal Interactions In Angiogenesis *Curr Opin Hematol*, 15 ,Pp. 204-209
- ❖ **Hunter, D.J., Willett, W.C. 1993.** Diet, body size, and breast
- ❖ **INDOX Cancer Research Network .2013.** Study "Retrieved 05-20
- ❖ **Insua-Rodríguez, J.; and Oskarsson, T.; 2016.** The extracellular matrix in breast cancer. *Adv Drug Deliv Rev.* 97:41-55.
- ❖ **Ioachim, E . ; et al. 2002** Immunohistochemical expression of extracellular matrix components tenascin, fibronectin, collagen type IV and laminin in breast cancer: their prognostic value and role in tumour invasion and progression, *Eur. J. Cancer* 38(18)2362–2370.
- ❖ **Iraqi Cancer Board .2004.** Results Of The Iraqi Cancer Registry Baghdad, Iraqi Cancer Registry Center, Ministry Of Health, (2007).
- ❖ **Iraqi national cancer research center . 2013.** Brief historical introduction, establishing the breast & cervical cancer research unit and the Iraqi National Cancer Research Center/Program
- ❖ **Ito, S. S.; Ishimaru, et al. 1998.** Effect of coacervated alpha-elastin on proliferation of vascular smooth muscle and endothelial cells. *Angiology* 49(4): 289-297.

- ❖ **James, w.d; Berger, t.g.; and Elston, d.m .2006.** Andrews' Diseases of the Skin: Clinical Dermatology (10th ed., p. 1), by Phila-delphia: Elsevier Saunders. Copyright 2006 by Elsevier Saunders. Reprinted with permission.
- ❖ **Jung, S.; Rutka, J. T.; and Hinek ,A. 1998.** Tropoelastin and elastin degradation products promote proliferation of human astrocytoma cell lines. *J. Neuropathol. Exp. Neurol.* 57 439–448. 10.1097/00005072-199805000-00007.
- ❖ **Kaaks, R.; Rinaldi,S.; and Key,T.J. et al.2005.** Postmenopausal Serum Androgens, Oestrogens And Breast Cancer Risk: The European Prospective Investigation Into Cancer And Nutrition. *Endocrrelat Cancer*;12(4):1071–1082.
- ❖ **Kadar, A.; Tőkés, A.M.; Kulka, J.; and Robert, L.2002.** Extracellular matrix components in breast carcinomas. *Semin Cancer Biol.* ;12:243–257. doi: 10.1016/S1044-579X(02)00027-5.
- ❖ **Kakarala, M.; Rozek, L.; Cote, M.; Liyanage, S. and Brenner, D.E.,. 2010.** Breast Cancer Histology And Receptor Status Characterization In Asian Indian And Pakistani Women In The US-A SEER Analysis . *BMC Cancer*.10:191 .
- ❖ **Kakarala, M.; Dean, E. and Brenner .2010.** in Progress in Molecular Biology and Translational Science.
- ❖ **Karnik, S. K.; Brooke, B. S.; Bayes-Genis,A.; Sorensen, L.; Wythe, J. D.; and Schwartz, R. S.; et al. 2003.**A critical role for elastin signaling in vascular morphogenesis and disease. *Development*130, 411–423. 10.1242/dev.00223.
- ❖ **Kelsey, J.L.; Gammon, M.D. and John E.M.1993.** Reproductive factors and breast cancer. *Epidemiol Rev* ;15:36-47.
- ❖ **Kerlikowske, K.; Shepherd, J.; Creasman, J.; Tice, J. A.; Ziv, E., and Cummings, S. R. 2005.** Are Breast Density And Bone Mineral Density Independent Risk Factors For Breast Cancer? *Journal Of The National Cancer Institute*, 97, 368-374
- ❖ **Key, T.; Appleby, P.; Barnes, I. and Reeves, G.2002.** Endogenous Sex Hormones And Breast Cancer In Postmenopausal

Women: Reanalysis Of Nine Prospective Studies. J Natl Cancer Inst;94(8):606–616. Carcinoma. Cancer Res 1985, 45:1428–1430.

- ❖ **Key, T.J., Verkasalo, P.K., and Banks, E. 2001.** Epidemiology Of
- ❖ **Kielty, C.M.; Sherratt, M.J. and Shuttleworth, C.A. 2002.** Elastic Fibres. J Cell Sci 115:2817–2828.
- ❖ **Kojima, Y. et al. 2010.** Autocrine TGF-Beta And Stromal Cell-Derived Factor-1 (SDF-1) Signaling Drives The Evolution Of Tumor-Promoting Mammary Stromal Myofibroblasts. Proc Natl Acad Sci U S A. ;107(46):20009–20014.
- ❖ **Kuhl .1996.** Effects Of Progestogens On Haemostasis . Maturitas.;24(1-2):1-19.
- ❖ **Kumar, G. L.; and Rudbeck, L.2009.** Education Guide, Immunohistochemical Staining Methods Fifth Edition . Dako North America, Carpinteria, California. USA. PP. 56-60.
- ❖ **Lapis, K. and Tímár, J.2002.** Role of elastin-matrix interactions in tumor progression. Semin Cancer Biol. 2002;12:209–217. doi: 10.1016/S1044-579X(02)00024-X.
- ❖ **Liotta, L.A . and Kohnthe, E.C.2001.** Microenvironment Of The Tumour-Host Interface Nature, 411, Pp. 375-379
- ❖ **Liu, X.; Zhao, Y.; Gao, J.; Pawlyk, B.; Starcher, B.; and Spencer, J.A.; et al.2004.** Elastic fiber homeostasis requires lysyl oxidase-like 1 protein. Nat Genet. 2004;36:178–82.
- ❖ **Lochter, A. and Bissel, M.1995.** Involvement Of Extracellular Matrix Constituents In Breast Cancer.
- ❖ **Love, Susan, M. 2015.** "1". Dr. Susan Love's Breast Book (6 ed.). U.S.A.: Da Capo Press. [ISBN 978-07382-1821-2](#).
- ❖ **Luna, L.C.1986.** Manual of histological staining methods of Armed forces .Insitute of pathology, 3 rded. McGraw-Hill Book.New York, London.9:1-74.

- ❖ **Madhuri, K.; Laura, R.; and Michele, C. *et al.*, 2010.**Breast cancer histology and receptor status characterization in Asian Indian and Pakistani women in the U.S.- a SEER analysis. *BMC Cancer* 2010, 10:191.
- ❖ **Magnusson, C.M.; Persson, I.R.; Baron, J.A.; Ekbom, A.; Bergstrom, R.; and Adami ,H.O.1999.**The role of reproductive factors and use of oral contraceptives in the aetiology of breast cancer in women aged 50 to 74 years. *Int J Cancer*. 1999;80:231–236.
- ❖ **Malone, K.E.; Daling,J.R.; Thompson, J.D.; O'Brien, C.A.; Francisco, L.V.; and Ostrander, E.A .1998.** "BRCA1 mutations and breast cancer in the general population: analyses in women before age 35 years and in women before age 45 years with first-degree family history". *JAMA*. 279 (12): 922–9.
- ❖ **Mantovani, A.; Allavena,p.; and Sica, A.; k.o *et al.*.2008.**Cancer-Related Inflammation *Nature*, 454 ,Pp. 436-444
- ❖ **Masters, J.R.; Sangster, k.; Hawkins, R.A.; and Shivas, A.A. 1976.**Elastosis and oestrogen receptors in human breast cancer. *Br J Cancer*. 1976;33:342–343. doi: 10.1038/bjc.1976.49.
- ❖ **Missmer, S.A.; Eliassen, A.H.; Barbieri, R.L.; and Hankinson, S.E. 2004.** Endogenous Estrogen, Androgen, And Progesterone Concentrations And Breast Cancer Risk Among Postmenopausal Women. *J Natl Cancer Inst*;96(24):1856–1865.
- ❖ **Mithieux, S. M.; and Rasko, E.J. *et al.* 2004.** Synthetic Elastin Hydrogels Derived From Massive Elastic Assemblies Of Self-Organized Human Protein Monomers. *Biomaterials* 25(20):4921-4927.
- ❖ **Mori, I.; Yang, Q.; and Kakudo, K.2002.** Predictive and prognostic markers for invasive breast cancer. *Pathol Int*, 52, 186-94 .

- ❖ **Nakamura, T. *et al.* 2002.** Fibulin-5/DANCE is essential for elastogenesis *in vivo*.
- ❖ **Nakanishi, I.; Moriizumi, T.; Ooi, A.; Oda, Y.; and Kajikawa, K. 1983.** An ultrastructural study on periductal elastosis in human breast tumors. *Acta Pathol Jpn.* 1983;33:761–772.
- ❖ **Nallasamy, S.; Yoshida, K.; Akins, M.; Myers, K.; Iozzo, R. and Mahendroo, M. 2017.** Steroid Hormones Are Key Modulators Of Tissue Mechanical Function Via Regulation Ofcollagen And Elastic Fibers. *Endocrinology* 158, 950–962.
- ❖ **National Cancer Institute .2014.**Cancer Treatment Breast ". Retrieved 29 June2014.
- ❖ **National Cancer Institute. SEER Cancer Statistics Review 1975-2009.** [Accessed 2015 Sept. 20] Available from:
- ❖ **National Cancer Institute.2009.** SEER Cancer Statistics Review 1975-2009. [Accessed 2015 Sept. 20] Available From: [Http://Seer.Cancer.Gov/Archive/Csr/1975_2009_Pops09/Results_Merged /Sect_04_Breast.Pd](http://seer.cancer.gov/archive/csr/1975_2009_pops09/results_merged/sect_04_breast.pdf)
- ❖ **Neubauer,N.L.; Ward, E.C.; Patel, P.; Lu, Z.; Lee, I.; Blok, L.J.; Hanifi-Moghaddam, P.; Schink, J. and Kim, J.J. 2011.** Progesterone Receptor-B Induction Of BIRC3 Protects Endometrial Cancer Cells From AP1-59-Mediated Apoptosis. *Horm. Cancer* 2,170-181.
- ❖ **Nisa,A .; Bhurgri, Y.;Raza ,F and Kayani ,N. 2008.**Comparison Of ER, PR And HER-2/Neu (C-Erb B 2) Reactivity Pattern With Histologic Grade, Tumor Size And Lymph Node Status In Breast Cancer. *Asian Pac J Cancer Prev*;9:553–6.
- ❖ **Oran, B.; Celik, I.;Erman, M.; Baltali, E.; Zengin, N.; Demirkazik, F and Tezcan, S. 2004.** Analysis Of Menstrual, Reproductive, And Life-Style Factors For Breast Cancer Risk In Turkish Women - A Case-Control Study. *Medical Oncology*, 21, 31-39.

- ❖ **Pamplona, D.C.; de Abreu, Alvim, C.2004.** Breast Reconstruction with Expanders and Implants: a Numerical Analysis. *Artificial Organs* 8 pp. 353–356.
- ❖ **Parkin, D.M and Fernandez, L.M. 2006.** Use Of Statistics To Assess The Global Burden Of Breast Cancer. *Breast*. 12(1 Suppl.):S70–S80.
- ❖ **Partridge, S. M. 1970.** in *Chemistry and Molecular Biology of the Intercellular Matrix* (edit. by Balazs, E. A.), 1, 593 (Academic Press, London, 1970).
- ❖ **Pittet,J.C.; Freis ,O.; Duchêne, M.D.V and Pauly,G.P.G. 2014.** Evaluation Of Elastin/Collagen Content In Human Dermis In-Vivo By Multiphoton Tomography—Variation With Depth And Correlation With Aging. *Cosmetics*.1, 211-221
- ❖ **Pourzand, A.; Fakhree, M.B.A.; Hashemzadeh,S.; Halimi,M.; and Daryani,A.2011.**Hormone Receptor Status In Breast Cancer And Its Relation To Age And Other Prognostic Factors. *Basic And Clinical Research*.
- ❖ **Rampaul, R. S.; Pinder, S. E.; Elaston, C.W.; and Ellis, I.O. 2001.** Prognostic and predictive factors in primary breast cancer and their role in patient management; the Nottingham breast team. *Eur J Surg Oncol*, 27, 229-38.
- ❖ **Rampaul, R.S.; Pinder, S.E.; Elaston, C.W.; and Ellis, I.O .2001.** Prognostic and predictive factors in primary breast cancer and their role in patient management; the Nottingham breast team. *Eur J Surg Oncol*, 27, 229-38.
- ❖ **Rasmussen, B.B.; Pedersen, B.V.; Thorpe, S.M. and Rose C .1985.** Elastosis In Relation To Prognosis In Primary Breast Carcinoma. *Cancer Res* 1985, 45:1428–1430.
- ❖ **Robert, L.1999.** Interaction between cells and elastin, the elastinreceptor.*Connect Tissue Res* 40:75–82.
- ❖ **Robertson, A. J.; Brown. R. A. S.; Cree, I. A.; McGillivray. J. B.; Slidders, W. and Swanson Beck, J.1981.**Prognostic Value Of

Measurement Of Elastosis In Breast Carcinoma. *J. Clin. Pathol.*, 34: 738-743.

- ❖ **Robinet, A.; Fahem, A.; Cauchard, J. H.; Huet, E.; Vincent, L.; and Lorimier, S.; et al. 2005.** Elastin-derived peptides enhance angiogenesis by promoting endothelial cell migration and tubulogenesis through upregulation of MT1-MMP. *J. Cell Sci.* 118 343–356. 10.1242/jcs.01613.
- ❖ **Rodgers, U. R., and Weiss, A. S. 2005.** Cellular interactions with elastin. *Pathol. Biol.* 53 390–398. 10.1016/j.patbio.2004.12.022.
- ❖ **Rodgers, U.R.; and Weiss, A.S. 2006.** Cellular Interactions With Elastin .
- ❖ **Rosenberg, L.U.; Einarsdóttir, K.; Friman, E.I.; Wedren, S.; Dickman, P.W.; and Hall, P. et al. 2006.** Risk Factors For Hormone Receptor-Defined Breast Cancer In Postmenopausal Women. *Cancer Epidemiol Biomarkers Prev*;15(12):2482–8.
- ❖ **Sage, H. and Gray, W. R. 1980.** Studies on the evolution of elastin. II. Histology. *Comp. Biochem. Physiol.* 66B, 13–22.
- ❖ **Sakakura T. 1991.** New Aspects Of Stroma-Parenchymal Relations In Mammary Gland Differentiation. *Int Rev Cytol* 125:165-202.
- ❖ **Samphao, S.; Wheeler, A.J.; Rafferty, E.; Michaelson, J.S.; Specht, M.C.; and Gadd, M.A, et al. 2009.** Diagnosis Of Breast Cancer In Women Age 40 And Younger: Delays In Diagnosis Result From Underuse Of Genetic Testing And Breast Imaging. *Am J Surg*;198(4):538-43.
- ❖ **Sator, P.G.; Schmidt, J.B.; and Rabe, T.; et al. 2004 .** Skin Aging And Sex Hormones In Women – Clinical Perspectives For Intervention By Hormone Replacement Therapy . *Exp Dermatol*, 13(Suppl 4):36-40.

- ❖ **Sator, P.G.; Schmidt, J.B; and Sator, M.O.; et al.2001.** The influence of hormone replacement therapy on skin ageing: a pilot study. *Maturitas*. 2001;39:43–55.
- ❖ **Senior, R. M.; Griffin, G. L.; and Mecham, R. P. 1982.** Chemotactic responses of fibroblasts to tropoelastin and elastin-derived peptides. *J. Clin. Investig.* 70 614–618. 10.1172/JCI110654
- ❖ **Setiawan,V.W.; Monroe, K.R.; and Wilkens,L.R.;et al.2009.** Breast Cancer Risk Factors Defined By Estrogen And Progesterone Receptor Status: The Multiethnic Cohort Study. *Am J Epidemiol*;169(10):1251–1259.
- ❖ **Shah, M.G.; and Maibach H.I. 2001 .**Esterogen And Skin: An Overview. *Am J Clin Dermatol*, 2:143-50.
- ❖ **Shelley, S.; Tworoger, ; Stacey ,A.; Missmer , Robert, L.; Barbieri , Walter C.; and Willett .2005.** Plasma Sex Hormone Concentrations And Subsequentrisk Of Breast Cancer Among Women Using Postmenopausal Hormones. *Journal Of The National Cancer Institute*, 97(8): 595 – 602.
- ❖ **Shikata, J.; Sanada, H.; Tamamuro, T. and Takeda, T.1979.** Experimental studies of the elastic fiber of the capsular ligament. *Connect lissue Res* :7:21-7.
- ❖ **Shin,H.J.; K.i.m, H.H.; Huh, M.O.;Kim M.J, Y.i.A.; Kim,H.; Son B.H. and Ahn, S.H.2011.** Correlation Between Mammographic And Sonographic Findings And Prognostic Factors In Patients With Node-Negative Invasive Breast Cancer. *Br J Radiol*.;84:19–30. Doi: 10.1259/Bjr/92960562
- ❖ **Shivas, A.A.; and Douglas, J.G.1972.** The prognostic significance of elastosis in breast carcinoma. *J R Coll Surg Edinb*. 1972;17:315–320.
- ❖ **Shubic, P . 1994.** Neoplasia: A General Pathological Reaction. *Cancer Lett* 83:3-7.

- ❖ **Sieri, S.; Krogh, V.; Bolelli, G.; et al.2009.** Sex Hormone Levels, Breast Cancer Risk, And Cancer Receptor Status In Postmenopausal Women: The ORDET Cohort. *Cancer Epidemiol Biomarkers Prev*;18(1):169–176.
- ❖ **Silvera, S. A. N.; Miller, A. B. and Rohan, T. E. 2005.** Oral Contraceptive Use And Risk Of Breast Cancer Among Women With A Family History Of Breast Cancer: A Prospective Cohort Study. *Cancer Causes & Control*, 16, 1059-1063.
- ❖ **Stevenson, S. And Thornton, J . 2007.**Effect Of Estrogens On Skin Aging And The Potential Role Of Serms. *Clinical Interventions In Aging*.2(3) 283-297.
- ❖ **Stöppler, Melissa,; Conrad. 2015.** . "[Breast Anatomy](#)". Retrieved 28 June 2015.
- ❖ **Strum, J. M.; Gartner, L. P.; and Hiatt, J. L. 2007.** Cell biology and histology. Hagerstown, MD: Lippincott Williams & Wilkins. p. 83.
- ❖ **Sughayer, MH.; AL-Kawaja, M.; Massarweh, S. and AL-Masri M .2006.** Prevalence Of Hormone Receptors And HER2 /Neu In Breast Cancer Cases In Jordan. *Pathol Oncol Res*; 12:83-6 .
- ❖ **Suvarchala, S. B. et al.,2011.** Carcinoma Breast – Histopathological And Hormonereceptor Correlation *Biosci Tech*, 2 (4):340-348 .
- ❖ **Tamura,S.; and Enjoji, M .1988.**Elastosis in neoplastic and non-neoplastic tissues from patient with mammary carcinoma. *Pathology international* , 38:12, P. 1537–1546.
- ❖ **Tworoger, S.S.; Missmer, S.A.; Barbieri, R.L.; Willett, W.C.; Colditz, G.A.; and Hankinson, S.E.2005.** Plasma sex hormone concentrations and subsequent risk of breast cancer among women using postmenopausal hormones.*J Natl Cancer Inst.* 2005 Apr 20;97(8):595-602.
- ❖ **Van Gils, C. H.; Hendriks, J.; Otten, J. D. M.; Holland, R. and Verbeek, A. L. M. 2000.** Parity And Mammographic Breast

Density In Relation To Breast Cancer Risk: Indication Of Interaction. European Journal Of Cancer Prevention, 9, 105-111

- ❖ **VanPutte, C.; Regan, J.; and Russo, A.2017.** Mammary Glands and Anatomy of the Breast - Female Reproductive System.
- ❖ **Viale , G. 2012.** Symposium Article. The Current State Of Breast Cancer Classification.; 23. 207-210.
- ❖ **Wagenseil, J.E. and Mecham.2007.** RP New Insights Into Elastic Fiber Assembly. Birth Defect Res C. 2007;81:229–240. Doi: 10.1002/Bdrc.20111.
- ❖ **Walker, M.2008.** Conquering Common Breast-Feeding Problems. J Perinat Neonatal Nurs; 22(4): 267-274.
- ❖ **Wastson, R.E.B.; Griffiths, C.E.M.; Craven, N.M.; Shuttleworth, C.A.; and Kielty, C.M .1999.** Fibrillin-Rich Microfibrils Are Reduced In Photoaged Skin. Distribution At The Dermal-Epidermal Junction. J Invest Dermatol 112:782-7.
- ❖ **Wendel, D.P.; Taylor, D.G.; Albertine, K.H.; and Keating M.T. L.i.D.Y .2000.** Impaired Distal Airway Development In Mice Lacking Elastin. Am J Respir Cell Mol Biol;23:320-6.
- ❖ **Wendel, D.P.; Taylor, D.G.; Albertine, K.H.; and Keating M.T. L.i.D.Y.2000.** Impaired Distal Airway Development In Mice Lacking Elastin. Am J Respir Cell Mol Biol;23:320-6.
- ❖ **Wennerberg ,K.; Lohikangas, L.; Gullberg, D.; Pfaff, M.; Johansson,S.; and Fassler R .1996.** α_1 Integrin-Dependent Polymerization Of Fibronectin. J Cell Biol ;132:227-38.
- ❖ **WHO. 2002.**Weight Control And Physical Activity. IARC
- ❖ **World Cancer Report, 2003.**World Health Organization pp chapter 10:23-231.
- ❖ **World Cancer Report. 2014.** World Health Organization. 2014.

Pp. Chapter 5.2.

- ❖ **Yamauchi, Y.; Tsuruga, E.; Nakashima, K.; Sawa, Y. and Ishikawa, H. 2010.** Fibulin-4 And -5, But Not Fibulin-2, Are Associated With Tropoelastin Deposition In Elastin-Producing Cell Culture. *Acta Histochem Cytochem* 43, 131-138.
- ❖ **Yanagisawa, H.; Davis, E. C.; Starcher, B. C.; Ouchi, T.; Yanagisawa, M.; Richardson, J. A. and Olsen, E. N. 2002.** Fibulin-5 is an elastin-binding protein essential for elastic fibre development in vivo. *Nature* 415, 168-171.
- ❖ **Yasui, Y. and Potter J.D.1999.** The Shape Of Age-Incidence Curves Of Female Breast Cancer By Hormone-Receptor Status. *Cancer Causes Control*199;10:431–7.
- ❖ **Youk , J. H. Gweon, H. M . Son , E.J. Kim , J.A.; and Jeong, J. 2013.**Shear-wave elastography of invasive breast cancer: correlation between quantitative mean elasticity value and immunohistochemical profile. *Breast Cancer Res Treat* 138:119–126
- ❖ **Zeleniuch-Jacquotte, A.; Shore, R.E.; Koenig, K.L. et al.2004.** Postmenopausal Levels Of Oestrogen, Androgen, And SHBG And Breast Cancer: Long-Term Results Of A Prospective Study. *Br J Cancer*.90(1):153–159.
- ❖ **Zhu ,X. ;Ying, J.;Wang ,F.Wang, J ; and Yang,H.2014.** Estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 status in invasive breast cancer: a 3,198cases study at National Cancer Center, China. 2014.*Breast Cancer Res Treat* 147:551–555.

الخلاصة

الغرض الرئيسي من هذه الدراسة هو التعبير عن البروتين المعروف بالاستين لكل من مستقبلات هرمون الاستروجين ER، مستقبلات هرمون البروجستيرون PR ومستقبل عامل نمو البشرة Her2. تم اجراء هذه الدراسة في كلية العلوم / قسم علوم الحياة.

بلغ العدد الكلي للنساء هو 60 عينة من سرطان الثدي المشخص القنوي الغازي وتم الحصول عليها من مختبرات مستشفى الحسين التعليمي / ذي قار، وكان متوسط عمر جميع المريضات هو 50 عاما، وكانت جميع النساء ضمن المرحلة الثالثة (3) stage والدرجة الثانية Grade (2).

كما و أظهرت مقاطع الجلد معدل عالي لتعبير بروتين الاستين والتي اعتمدت كعينات سيطرة control.

أشارت النتائج إلى وجود ارتباط ايجابي معنوي عالي بين تعبير مستقبل بروتين الاستين والعمر وكان الارتباط المعنوي بمعدل (0.45) عند مستوى احتمال $P < 0.05$ ، كما أثبتت هذه الدراسة أيضا وجود ارتباط ايجابي معنوي عند مستوى احتمال $P < 0.05$ بين العمر وتعبير مستقبلات ER+, PR+ اذ كان قيمة الارتباط 0.235 و 0.245 على التوالي.

كما أظهرت هذه الدراسة وجود علاقة ارتباط معنوية عند مستوى احتمالية $p < 0.05$ بين التعبير عن مستقبلات بروتين الاستين ومستقبلات ER و PR الموجبة 0.312 و 0.355 .

بينت النتائج الإحصائية أيضا وجود ارتباط سلبي غير معنوي بلغت المعدلات (0.038 - و -0.299) على التوالي لكل من هرموني PR و ER ، فضلا عن وجود ارتباط قوي ايجابي بين التعبير عن مستقبلات PR ، ER عند مستوى احتمال $p < 0.01$ اذ بلغت قيمة هذا الارتباط 0.541.



جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة ذي قار

كلية العلوم

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من قبل

لمياء علي كاظم السعداوي

بكالوريوس علوم-علوم حياة

2013

بإشراف

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